

BIOCHEMICAL AND PHARMACOLOGICAL STUDIES
OF AMYGDALOID KINDLING IN THE RAT

BY

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"A convulsion is but a symptom and implies only that there is an occasional, an excessive, and a disorderly discharge of nerve tissue on muscles. This discharge occurs in all degrees; it occurs with all sorts of conditions of ill-health, at all ages, and under innumerable circumstances.....I trust I am studying the general subject of convulsion methodically when I work at the simplest varieties of occasional spasm I can find."

JOHN HUGHLINGS JACKSON

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MATERIALS

The animals used for these experiments were male albino Wistar rats bought from the University of Edinburgh Centre for Laboratory Animals.

Non-radioactive reagents were obtained from British Drug Houses Ltd., Poole, England (Analar reagent grade) and radioactive substances were bought from Radiochemical Ltd., Amersham, England, unless otherwise stated.

ABBREVIATIONS

SI Units were used throughout as outlined in Biochemical Journal (1978) 169, 1 - 27. The following abbreviations have also been used:

ACE	acetylcholinesterase	EC 3.1.1.7
ATP	adenosine triphosphate	
ATPase	adenosine triphosphatase	EC 3.6.1.4
COMT	catecholamine-o-methyltrans-ferase	EC 2.1.1.a
DOG	2-deoxy-D-glucose	
DOG-6P	2-deoxy-D-glucose-6-phosphate	
ECS	electroconvulsive shock	
ECT	electroconvulsive therapy	
EDTA	ethylene-diaminetetracetate	
GABA	γ -aminobutyric acid	
5-HT	5-hydroxytryptamine	
cyclic-GMP	3', 5'-cyclic guanine monophosphate	
I. P.	intraperitoneal	
I. V.	intravenous	
Pi	orthophosphate (inorganic)	
PPO	2, 5-diphenyloxazole	
POPOP	1, 4-Di 2-(5-phenyloxazolyl) benzene	
LSR	least significant range	
rev/min (rpm)	revolutions per minute	
cpm	counts per minute	
dpm	disintegrations per minute	
Tris	2-amino-2-hydroxymethylpropane-1, 3-diol	

Dopamine

3, 4-dihydroxyphenylalanine

TOH

tyrosine hydroxylase

QNB

quinucleodinyI benzilate

STATEMENT IN TERMS OF REGULATION 2.4.15 AND 2.4.11 OF THE
POSTGRADUATE BOARD OF STUDY PROGRAMME OF THE UNIVERSITY
OF EDINBURGH

- (a) I declare that I composed this thesis.
- (b) The experimental work is my own with the following exceptions:
1. Throughout this project a major contribution to the preparation of electrodes and the operating and stimulating procedures was made by Mr. M. Martin, who assisted with the histological techniques and also performed the assay of ACE.
 2. The measurement of cyclic GMP was done together with Dr. T. Palomo.
 3. Mr. V. Kapoor collaborated in the experiments using deoxy-glucose and performed some of the dissections contributing to the data presented in chapter 4.
 4. Mrs. A. Wright kindly prepared the enzyme catecholamine-o-methyl-transferase used for the assay of dopamine and norendrenaline.

The following publications are based on work described in this thesis:

1. Blackwood, D.H.R., Kapoor, V. and McQueen, J.K.
Regional changes in cerebral glucose utilization in kindled rats during convulsions.
British Journal of Pharmacology 68 (1), 1980, 133P.
2. Blackwood, D.H.R.
Amygdaloid Kindling in Rats: Neurochemical and Pharmacological Studies.
In "Animal models of neurological diseases".
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ABSTRACT OF THESIS

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Title of Thesis Biochemical and Pharmacological Studies of Amygdaloid Kindling in the Rat

The kindling effect was investigated in the rat amygdala in an attempt to identify the biochemical correlates of the long-lasting changes in neuronal excitability which are characteristic of this phenomenon. The hypothesis that cholinergic mechanisms are facilitatory and catecholaminergic systems are inhibitory in amygdaloid kindling was tested. The cholinergic muscarinic antagonist atropine was found to be without effect on kindling and muscarinic receptor sites and sodium-dependent, high affinity choline uptake were not perturbed one month after a kindled convulsion, even though the epileptogenic effect of kindling is known to persist for over 1 year. From these results it was concluded that there was not a significant cholinergic contribution to the development or maintenance of kindling.

The involvement of noradrenaline and dopamine in kindling was assessed by measuring the turnover rates of these amines. At the site of stimulus the basal level of noradrenaline was found to be reduced and the rate of depletion of dopamine was increased when compared with the contralateral unstimulated amygdala. Cyclic GMP levels in slices prepared from the amygdala was higher in kindled than in sham-operated animals, whilst an impaired response of cyclic GMP to depolarization or to media containing dopamine or haloperidol was recorded. These findings suggest a role for catecholamines in kindling and support the view that the long-term changes in kindling are associated with dopamine receptor subsensitivity accompanied by a compensating increase in presynaptic dopamine release.

During the course of these experiments it became apparent that the very presence of metal electrodes in the amygdala, for periods of up to 4 weeks, caused enhancement in the rate of kindling. This observation, which has some implications for theories about kindling, was confirmed using both stainless steel and platinum/iridium metal implants.

Finally, a comparison of regional metabolic requirements during kindling induced convulsions, electroshock convulsions and partial kindling were compared and contrasted with regional requirements in sham-operated and unoperated control *ANIMALS* using a deoxy-glucose technique for estimating regional glucose utilization.

Changes observed in glucose consumption were always bilateral and in no area did rats which had been kindled but not convulsed at the time of the deoxyglucose experiment, differ from sham operated controls. In two regions the hypothalamus and septal nuclei unoperated controls showed a significantly lower uptake than in sham operated rats. Since these groups differed only in the placement of an electrode into the amygdala the results were taken to imply that the presence of an electrode caused increased neuronal activity in those regions. Kindled induced convulsions were associated with an increase in neuronal activity in the amygdalae, hippocampi, superior colliculi, substantia nigra and septal nuclei. Partial kindling caused increased glucose consumption in the amygdalae hippocampi and superior colliculi. Electro-convulsions led to increased neuronal activity in the amygdalae, hippocampi, hypothalami, substantia nigra, septal nuclei thalamus, striata, cerebellar nuclei, cerebellar hemispheres and reticular formation. Theories about the anatomy of spread of epileptic seizures are discussed.

CHAPTER 1

INTRODUCTION

1 - 1 SCOPE OF THE INTRODUCTION

This chapter is intended as an up-to-date review of all aspects of the topic of kindling, and is not limited only to the material available when the experiments described in this thesis were planned and carried out.

As is inevitable in a rapidly evolving area of study, several important observations published during the progress of my work variously conflicted or were in agreement with my own conclusions. For the sake of clarity and completeness, publications up to March, 1980 are included in this review and points of comparison or contrast with the experimental results presented in this thesis are developed in the appropriate discussion sections of each chapter.

1 - 2 BACKGROUND

The neuronal mechanisms underlying the enhanced behavioural response observed in organisms following the continued application of a stimulus have long been the subject of study by neurophysiologists interested in the mechanisms of learning and memory. The phenomenon of post-tetanic potentiation, defined by Eccles (1953) as "an increased postsynaptic discharge elicited homosynaptically and due to increased presynaptic action", has long been known to be a fairly generalized effect and has been observed in a large number of neural junctions (Hughes, 1958), but its effects, which persist for minutes or hours, are generally short-lasting. A more enduring neuronal response to repeated stimulation emerged from studies of experimental epilepsy. Morrell (1961) had demonstrated that chronic irritative focus in the brain may lead to both the local development of a focus of epileptic activity as well as to a more distant secondary focus. The development of a secondary focus was usually both transynaptic and slow (requiring days to months) and believed to be the outcome of continuous synchronous bombardment from one epileptic zone to a related non-epileptic area (Morrell, 1969). Greater flexibility and control over the parameters of stimulation can be obtained by using electrical stimulation in the place of chemical irritants. Delgado and Sevillano (1961) showed that repeated electrical stimulation applied to the hippocampus evoked a progressive increase in convulsive behaviour and

ultimately in a generalized seizure. However, Goddard (1967) and Goddard et al (1969) were the first people to recognize the possible physiological and behavioural significance of the observation that spaced and repeated epileptogenic stimulations applied to certain brain regions could cause an apparently permanent alteration in neural connectivity. To describe this effect he coined the term "kindling".

1 - 3 THE KINDLING EFFECT

The kindling effect has been exhaustively reviewed by Racine (1978). The general procedure described by Goddard et al (1969) has been followed by most other workers in the field, with few modifications. Goddard used as subjects rats, cats and monkeys and, in a typical experiment in the rat, bipolar stainless steel nichrome or platinum electrodes, insulated with diamel or teflon, were stereotaxically implanted under anaesthesia into the amygdala nucleus and anchored to the skull with stainless steel screws and dental acrylic. One week after surgery daily stimulation was begun with biphasic 1 millisecond pulses at a rate of 62.5 Hz, delivered for a duration of 1 second at an intensity of 50 μ a peak to peak. After the first few stimulation trials, the EEG recorded no after-discharge and the animal showed no behavioural change. At about the seventh trial an after-discharge was evoked by the stimulus and the animal responded by eye closure, movement arrest and chewing movements. From this state onwards the after-discharge which followed each stimulus became progressively longer and the accompanying behavioural changes became bilateral, until at about the fourteenth stimulus the first bilateral clonic convulsion was observed. Convulsions could then be reliably triggered on each successive day, but spontaneous convulsions between stimulations did not occur. Racine (1972a and b) extended the work of Goddard by stressing the importance of the after-discharge in kindling. He first demonstrated that repeated subthreshold stimulation did have the effect of lowering the after-discharge threshold, but did not have a kindling effect. The rate of kindling should, therefore, be measured as the number of after-discharges evoked and not as the number of stimulations applied. Racine also codified the behavioural changes which accompanied kindling in rats, introducing a scheme which has been almost universally adopted by other authors. The motor seizures were rated into five classes:

Stage I: Mouth and facial movements.

Stage II: Head nodding.

Stage III: Forelimb clonus.

Stage IV: Rearing.

Stage V: Rearing and falling.

By this scheme a full generalized seizure with loss of postural control is referred to as "a Stage or Class V motor seizure". Subsequently it was shown by other workers that when kindling stimulation is applied continuously over a long period (several hundred stimulations) there is a progressive intensification of motor seizures and a development of an epileptic syndrome characterized by spontaneously recurring seizures. Spontaneous seizures have been observed in rats (Pinel et al, 1975, Pinel and Rovner, 1978), cats (Wada et al, 1974a) and baboons (Wada et al, 1975, Wada and Osawa, 1976).

Pinel and Rovner (1978) systematically investigated the development of spontaneous epilepsy in kindling and extended Racine's behavioural classification of motor seizures as follows:

Stage VI: Multiple rearing and falling episodes.

Stage VII: A running fit.

Stage VIII: A running fit with periods of myoclonus.

He found that long-term kindling stimulation of the hippocampus, caudate, entorhinal cortex and amygdala invariably caused spontaneous seizures in rats and these brain regions did not differ significantly either in the form of convulsions induced or in the number of stimulations required to induce seizures. Although spontaneous seizures only occur after prolonged kindling, spontaneous interictal spiking is a constant feature during all stages of kindling in the rat and other species (Morrell and Tsuru, 1976, Pinel and Rovner, 1978, Fitz and McNamara, 1979a). In a recent study in the rat the latter noted that interictal spiking developed first from the site of stimulus and rapidly spread to other limbic structures bilaterally. At the completion of kindling most interictal spikes involved the limbic structures, especially the amygdala and hippocampus, in a bilaterally synchronous pattern with only occasional involvement of the reticular formation or neocortex. Thus the

distribution of interictal spiking, which was not observed in unstimulated rats up to 10 days post-operatively, may provide an indication as to which regions develop enhanced neuronal excitability in kindling.

Reference has already been made to kindling in rats, cats, monkeys and baboons. Kindling has also been demonstrated in the frog (Morrell and Tsuru, 1976), mouse (Leach and McIntyre, 1976) and rabbit (Tanaka, 1972). Wada (1978) has directed attention to the striking similarities in the pattern of convulsive behaviour among different species. He proposed that the lower the organism is in the phylogenetic scale, the easier it is to reach the stage of generalized convulsions by amygdaloid kindling. Furthermore, in all species investigated kindling can be more readily achieved from the amygdala than from the cortex. He suggested that in man kindling may only occur in patients with genetic or other predisposition to epilepsy.

1 - 4 THE NATURE OF THE STIMULUS REQUIRED TO EVOKE KINDLING

(a) Electrical Stimulation

Racine (1972b), having stressed the critical importance of after-discharge production in kindling, found that within fairly wide limits other parameters of electrical stimulation are not critical. Goddard (1969) produced a kindling effect using sine wave, capacitatively coupled rectangular pulses and biphasic rectangular pulses. Most workers have used biphasic square wave pulses, but Racine et al (1973a) found no difference in the effect of monophasic and biphasic stimulation for triggering an after-discharge and motor seizures from the amygdala. In our own experiments, on account of the limitations of equipment available, we have used monophasic square wave pulses as a stimulus.

Goddard found a remarkable independence of stimulus parameters and the rate of kindling from the amygdala in rats. The time to first bilateral convulsion remained 10 to 15 days for a range of stimulus intensities of 50 μ a to 10 ma, when the stimulus was applied either for 1 second or for 60 seconds daily. Similarly, there was no difference in the number of days to first convulsion when using frequencies of 25 Hz, 60 Hz or 150 Hz, or when sine wave or 1 millisecond rectangular pulses were used. However, a frequency of 60 Hz was found to be optimal for eliciting a seizure in rats after kindling had become fully established. Contrastingly, the interval between stimu-

ations was found to be of critical importance. In an experiment by Goddard a group of rats received continuous stimulation for two to three days without interruption, while other groups received 60-second bursts, separated by 5, 10 or 20 minutes or by 8, 12 or 24 hours. A final group received a stimulus for 60 seconds every seventh day. Massed trial stimulation involving intervals of 10 minutes or less resulted in adaptation and complete kindling did not occur, although subsequent kindling by daily stimulation was enhanced. For longer intervals between stimulation the number of trials to first convulsion varied with the interval between each trial, the optimum gap being 24 hours. There was no difference between stimulus, once in 24 hours and once in 7 days. However, Goddard had not recognized the importance of evoked after-discharge in kindling and this was not recorded in his experiments. Racine et al (1973b), using stimulations which reliably evoked after-discharge and testing inter-trial intervals of 15 minutes, 30 minutes, 1 hour, 2 hours and 24 hours, found that 2 hours was the minimum interval for optimum kindling.

(b) Chemical Kindling

The possible clinical relevance of kindling is suggested by a number of reports of the kindling effect produced by the repeated application of a number of pharmacological agents. The daily injection into the amygdala, hippocampus or caudate of carbachol caused a sequence of changes in rats which closely mimicked the effect of kindling by daily electrical stimulation (Vossu and Wise, 1975). Repeated administration of the convulsant agent pentylenetetrazol (Mason and Cooper, 1972, Pinel and Cheung, 1977) in a dose which is initially subconvulsive, given at intervals of over forty-eight hours, eventually caused convulsions and lowered convulsive threshold. A similar progressive and enduring enhancement in response following repeated spaced administration of a drug has been observed with: cocaine (Post and Kopanda, 1976), lidocaine (Post et al, 1975) and chlordimeform (Yim et al, 1977). Repeated electroshock to rats, provided convulsions are induced at intervals of not less than 24 hours, is also reported to lead to a gradual intensification of evoked seizures (Ramer and Pinel, 1976). Oliver et al (1979) have found evidence that five daily injections of pentylenetetrazol to guinea pigs causes long-term changes in the excitability of the hippocampus, which can be tested in slice preparations. Hippocampal slices generated epileptiform activity even when the animals were

killed long after the cessation of drug administration.

Although a unifying hypothesis is appealing, in the absence of electrophysiological data and knowledge of the anatomical site of the action of these drugs, caution must be exercised in the interpretation of "chemical kindling". Kindling has been defined fairly strictly in terms of behavioural and electroencephalographic changes and there are some indications that the behavioural response of animals to drugs may be operated by mechanisms other than those which operated during electrical kindling. Recently, for example, Rackham and Wise (1979) have shown that cocaine sensitization and amygdaloid kindling are independent phenomena, since prior kindling by repeated cocaine injections did not shorten the number of stimulations required for kindling by electrical stimulation. Furthermore, Ito et al (1977) showed that phenobarbitone and phenytoin in doses which blocked electrically kindled convulsions did not prevent convulsion in rats kindled by repeated injections of pentetrazol.

1 - 5 LOCAL HISTOLOGICAL CHANGE IN KINDLING

It has been claimed that the implantation of electrodes in the brain involves minimal and functionally-insignificant cell damage limited to the path of the insertion (Delgado, 1977) and that kindling is not a consequence of tissue damage to cells in the stimulated region, but is the consequence only of the increased activity of these cells under near-physiological conditions. Goddard et al (1969) ruled out an effect of oedema, gliosis or vascular changes or other reactions to the physical presence of the electrode, on the basis that these effects would be time-dependent and convulsions based on this type of change should appear at a fixed time after surgery. Furthermore, electrodes constructed from four different types of insulator (diamel, insl-x, epoxylite and teflon) and three types of metal (nichrome, stainless steel and platinum) were used and no differences in the rates of kindling were observed, even though the response of brain tissue to these metals differs. However, the numbers of rats tested were very small and, as Goddard at that time applied subthreshold stimulation for kindling, his measurement of kindling rates may have been inaccurate. Racine et al (1976) reported a Golgi study of pyramidal cells in the rat cortex at the site of stimulation which showed no alteration in cell size, dendritic branching or in the number or size of dendritic spines. Goddard et al (1969) examined the tissue in the stimulated amygdala using

Weil's staining for myelin and thionine stain for nissl substance. Electron microscopic studies by Racine and Zaide (1978) in the cortex and by Goddard and Douglas (1975) in the amygdala demonstrated no degenerative effect. Brotschi et al (1978) reported no astrocytic change at the kindling focus comparable to changes seen at a cobalt focus. However, although it remains undisputed that the minimal damage caused by implanting an electrode is not sufficient to cause kindling, it would be wrong from this evidence to claim that the presence of an electrode does not contribute in some way to the kindling process, by acting as a chronic irritative focus. A test of the effect of electrode implantation is to delay the time after operation at which the kindling procedure is begun and this essential control experiment is not recorded in the published literature on kindling. Chapter 3 describes such an experiment.

1 - 6 THE ANATOMY OF KINDLING

(a) The Anatomy of the Rat Amygdala

The following descriptions of experiments dealing with the anatomy of kindling will be made more clear by a brief review of the anatomical connections of the amygdala in the rat.

Recent work by Krettek and Price (1977ab, 1978ab) has provided a detailed description of the afferent and efferent projections of the amygdala in the rat. Each nucleus in the amygdala complex supports an entirely different set of connections with other brain structures and even when two nuclei send fibres to the same regions the terminations have a strictly laminated pattern, suggesting that the functions of each nucleus must be considered separately.

The amygdala has direct connections with the olfactory system, the cortex, the thalamus, hypothalamus and brain stem structures. Efferents also pass directly to the contralateral amygdala and hypothalamus.

Olfaction

Input from the primary olfactory system arises in the olfactory bulb, projects through the lateral olfactory tract and terminates in the cortical amygdaloid nucleus. Accessory olfactory inputs to the lateral basal nuclei also arise in the prepiriform and periamygdaloid cortex.

Hypothalamus/Midbrain and Brain Stem

Fibres of the stria terminalis and the diffuse ventral amygdalofugal pathway from reciprocating connections between the amygdala and the

bed nucleus of stria terminalis, the preoptic nucleus and the ventromedial nucleus of hypothalamus and additional fibres pass to the lateral septal nucleus, nucleus accumbens, the diagonal band and the median forebrain bundle, thus securing widespread direct connections between the olfactory system and midbrain and brain stem structures.

Commissural

The stria terminalis also carries a crossed component of fibres which traverses the anterior commissure and terminates in the contralateral bed nucleus of the stria terminalis and contralateral cortical and medial nuclei of the amygdala.

Thalamus

Fibres originating in the basolateral and lateral amygdaloid nuclei follow the course of the ventral amygdalofugal pathway to terminate bilaterally in the dorsomedial and habenular nuclei of the thalamus.

Cortex

Projections from the basolateral and lateral nuclei of the amygdala to the neocortex terminate in two distinct areas. One region is on the lateral surface of the hemisphere just dorsal to the rhinal sulcus and the second projection is to the medial surface of the frontal pole rostral and ventral to the genu of the corpus callosum.

Brain Stem

The central nucleus of the amygdala sends fibres to widespread areas of the ipsilateral mesencephalic tegmentum, in particular the bulbar reticular formation and to the pontine and medullary tegmentum. These fibres are thought to travel via the ventral amygdalofugal pathway and not by way of the stria terminalis.

(b) Rate of Kindling from Various Brain Sites

Goddard (1969) investigated the anatomical specificity of kindling and found very large differences between brain regions in their propensity to kindle. In decreasing order of ease of kindling were: amygdala (15), globus pallidus (22), pyriform cortex (24), olfactory area (29), entorhinal cortex (37), olfactory bulb (44), septal area (55), preoptic area (63), caudate/putamen (74), hippocampus (77). The number in brackets represents the mean number of

trials to first convulsions for all electrodes in that region. Goddard proposed a rough relation between the rate of kindling and the extent of anatomical connection with the amygdala. Areas which did not kindle even after 200 stimulations included the red nucleus, substantia nigra and cerebellum, reticular formation, central grey matter, ventral tegmental nucleus and the tectum. However, as previously noted, Goddard was not necessarily using above-threshold stimulation intensities and the variation between the brain regions which he recorded possibly merely reflects the differences in after-discharge threshold. Thus Pinel and Rovner (1978), using above-threshold stimulation, observed no difference in the rate of kindling from the amygdala, hippocampus, entorhinal cortex and caudate. Additionally, Cain and Corcoran (1978) found the rate of kindling from the olfactory bulb was faster than from the amygdala, although the threshold for after-discharge in the olfactory bulb was also higher. Racine (1978) reported the development of seizures by posterior neocortical kindling. Cain and Corcoran (1978) observed that in the olfactory system kindling from the olfactory bulb was dependent upon the amygdala and other limbic areas. Thus it seems that the olfactory bulb, amygdala and other limbic structures may act as a unit in the production of after-discharge. Le Galle La Salle (1979) found that the bed nucleus of the stria terminalis had a susceptibility to kindling second only to that of the amygdala and the features of kindling were similar to, but not dependent on, amygdaloid kindling. Thus bilateral amygdalotomy did not influence kindling of this nucleus. These observations are in keeping with Goddard's contention that the ease of kindling in different limbic structures is related to the degree of anatomical connection with the amygdala, a view further supported by the mapping of kindling facility in different regions of the hypothalamus (Cullen and Goddard, 1975).

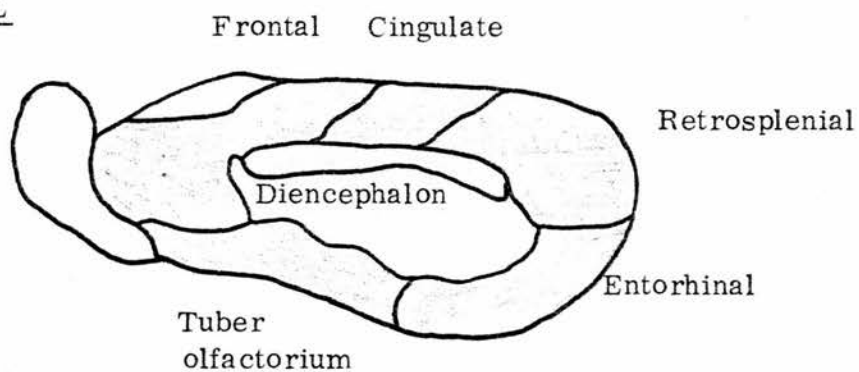
(c) Role of Cortex in Kindling

Racine (1975) applied kindling stimulation to several regions of the rat cortex. Three general types of response were obtained. From paleocortical areas (piriform cortex, entorhinal cortex) the electrographic features and behavioural seizures were similar to the developments which resulted from amygdala stimulation. Stimulation by electrodes in anterior neocortical regions evoked quite a different response. The first after-discharge was accompanied by a behavioural response which was usually a mild motor seizure involving face and mouth movements and myoclonus of the forelimbs.

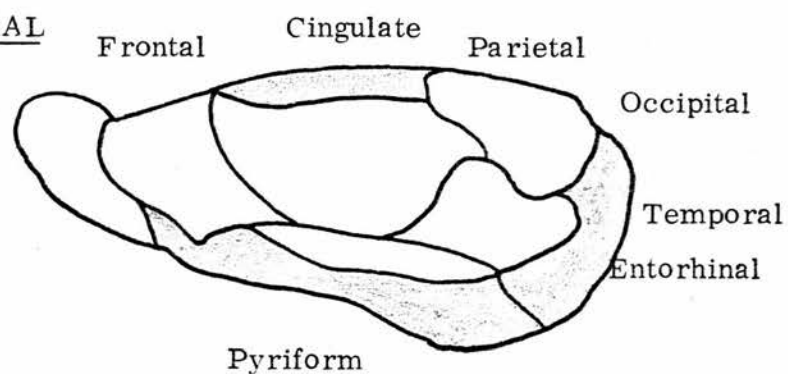
Repeated stimulation increased the strength of the seizures, which became generalized, but which nevertheless remained distinct from the type of seizure observed after limbic stimulation. Posterior neocortex stimulation caused electrographic changes indistinguishable from the anterior cortex stimulation, but the animals showed no signs of a behavioural seizure response. Initial after-discharge thresholds were lower in paleocortical than neocortical sites. Electrographic and behavioural responses from the cingulate cortex and frontal pole were intermediate between paleo and neocortical response. The difference in response to kindling may be reflected in the anatomy. The paleocortex is directly linked to the amygdala (Krettek and Price, 1974), whereas the neocortex does not appear to project to the amygdala (Gloor, 1978). The role of the cortex in amygdaloid kindling in rats was further investigated by Corcoran et al (1976). Applying kindling stimulation unilaterally to the amygdala, the electrographic response of the motor cortex was recorded. The development of kindled amygdala seizures was generally found to be correlated with the development of after-discharge activity recorded from the motor cortex, but the coincidence was not very close. In a few animals, for example, clinical seizure manifestations were observed before an after-discharge was recorded from the motor cortex. In further experiments bilateral lesions in the motor and premotor cortex, the same areas from which electrographic recordings had been made in earlier experiments, did not affect the rate at which rats developed amygdaloid convulsions. The ineffectiveness of lesions of the motor cortex to affect kindling suggests that this area is not critically involved. Lesions in the cingulate cortex and posterior neocortex similarly did not affect the rate of subsequent amygdaloid kindling, the latter stages of which were, however, significantly retarded by lesions of the paleocortex in the region of the rhinal sulcus, and large lesions involving the prefrontal cortex. This again fits precisely with the anatomic projections of the amygdala (Krettek and Price, 1974). The results of kindling stimulation applied to various cortical regions and the effects of cortical lesions in the cat closely resemble findings in the rat, confirming the conclusion that parts of the frontal cortex and the entorhinal cortex participate in, but are not essential for amygdaloid seizure development (Wake and Wada, 1976, Wada and Wake, 1977).

THE TELENCEPHALIC CORTEX PARCELLATED

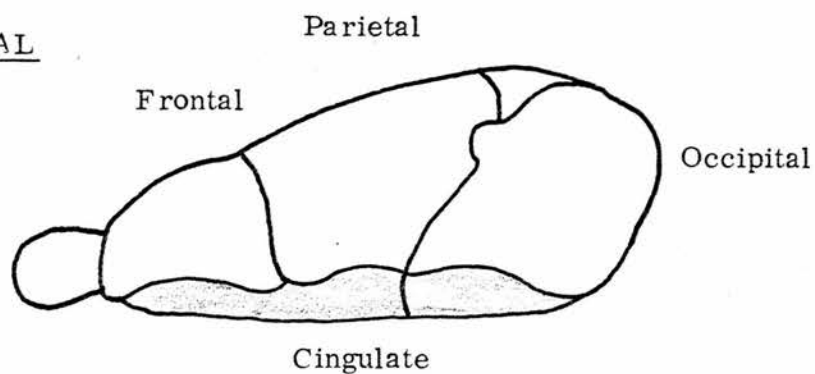
MEDIAL



LATERAL



DORSAL



(REDRAWN FROM 'CRAIGIE'S NEUROANATOMY OF THE RAT',
ACADEMIC PRESS, 1963)

(d) Role of the Thalamus in Kindling

Lesions of the inferior thalamic peduncle, rostral thalamic nuclei, dorsomedial nucleus and ventroanterior nucleus did not retard the rate of kindling and did not cause any change in the after-discharge morphology or duration (McCaughran et al, 1978a). However, in cats Stermann et al (1979) reported marked inhibition of kindling from the amygdala, following a lesion of the ventrobasal thalamus, which led them to conclude that sensorimotor thalamic circuits were involved in the conduction of subcortical seizure activity.

Recently, Cain (1979) has proved that chronic stimulation of a number of thalamic sensory nuclei will eventually produce a kindling response, which is quite dissimilar in appearance to seizures induced by limbic kindling and which is probably due to intrinsic changes in the sensory system rather than to spread of stimulus to limbic structures. Kindling of sensory systems is of considerable theoretical and clinical significance. However, the contrasts with limbic kindling reinforce the view that any effect of the thalamus on amygdala kindling is likely to be an indirect rather than a direct one.

(e) Role of Olfactory Inputs to Amygdala in Kindling

Rats with ablation of the olfactory bulb kindled normally from the amygdala (Cain and Corcoran, 1978). Large lesions which included the olfactory peduncle and which may have damaged the stria terminalis facilitated kindling from the amygdala.

(f) Role of Brain Stem Structures in Kindling

Using cats, Wada and Sata (1974 and 1975a) observed that commissurotomy disrupted the propagation of after-discharge to the contralateral amygdala, but not to the midbrain reticular formation, where bilateral after-discharges were reliably evoked at all stages of kindling, regardless of the extent of forebrain dissection. Wada proposed that the development of generalized seizures from the amygdala was by vertical propagation into the brain stem along extrapyramidal pathways, and that the establishment of a bilaterally synchronous autonomous focus of after-discharge in the reticular formation was a prerequisite for the development of a symmetrical bilateral convulsion. His views received added support when he demonstrated that an electrolytic lesion in the midbrain reticular formation, ipsilateral to the stimulated

amygdala, elevated the seizure threshold and reduced the susceptibility to pentylenetetrazol-induced convulsions (Wada and Sata, 1975b). Systematic mapping by depth electrodes of the brain stem has not been performed and, although Wada has stressed the pivotal role of the midbrain reticular formation, other neighbouring structures may be equally important in the propagation of kindling, since Wada employed fairly extensive lesions.

(g) Role of the Forebrain Commissures

Several investigators have found both in cats (Wada and Sata, 1975a) and in rats (McIntyre, 1976, McCaughran et al, 1977, McCaughran et al, 1978a, Nobrega and Gaito, 1978) that the rate of kindling is enhanced from the amygdala if the corpus callosum, hippocampal commissure and anterior commissure are sectioned before kindling stimulation is applied. Furthermore, when kindling reaches completion, generalized seizures are asymmetrical and predominantly affect the face and the body and limbs contralateral to the stimulus site.

In split brain rats, when kindling from one amygdala is followed by stimulation from the contralateral amygdala, the second site also generates only unilateral seizures. Effects on the rate, morphology and duration of seizures seem to occur only when all callosal and commissural connections are severed. Partial commissurotomy may be without effect and in particular there is no evidence for a special role for the anterior commissure in kindling.

Taken together with the results of experiments described above on the role of brain stem structures in kindling, it is now possible to conclude:

1. Interhemispheric connections probably exert a suppressive action on the kindling process (Nobrega and Gaito, 1978).
2. Intact interhemispheric connections are essential for the bilateral symmetry of behavioural convulsions, at least in the early stages.
3. Brain stem structures are critical for the bilateral spread of after-discharge and the establishment of kindling changes in the contralateral hemisphere. The midbrain reticular formation may not necessarily be directly in the path of seizure propagation, but could be responsible for enhancing amygdaloid seizure mechanisms by

ascending pathways.

(h) Conclusion

By the use of stimulation, depth electrodes and lesioning experiments many investigators have endeavoured to identify the anatomical sites which are critically involved in kindling from the amygdala. The evidence which has been summarized suggests that the olfactory, thalamic, cortical and interhemispheric commissural connections of the amygdala are not critically involved in the development of full generalized seizure by amygdaloid kindling. At the most, lesions of these sites may cause a delay in the rate of kindling and a temporary bilateral asymmetry in seizure manifestations, but will not inhibit its development. On the other hand, amygdala after-discharge appears to propagate synchronously to brain stem structures, however incomplete the interhemispheric connections. Table 1 summarizes the effect of knife cut lesions to various rat and cat brain areas on amygdaloid kindling.

TABLE I

THE EFFECT OF LESIONS IN VARIOUS BRAIN REGIONS
ON AMYGDALOID KINDLING IN RATS AND CATS

Site of Lesion	EFFECT				Reference
	Bilateral or Ipsilateral to Stimulus	Rate of Kindling	Type of Kindled Seizure		
<u>CORTEX</u> (Rat and Cat)					
Orbital	Bilateral	Slower	Normal		1,12
Pre-Frontal	"	"	"		"
Motor	"	Normal	"		"
Pre-Motor	"	"	"		"
Anterior Cingulate	"	"	"		"
Posterior Neocortex	"	"	"		"
<u>THALAMUS</u> (Rat)					
Inferior Peduncle	Bilateral	Normal	Normal		2, "
Rostral Nucleus	"	"	"		"
Dorsomedial Nucleus	"	"	"		"
Ventro Anterior	"	"	"		"
<u>OLFACTORY BULB</u> (Rat)	Ipsilateral	Normal	Normal		3,

TABLE 1 (continued)

THE EFFECT OF LESIONS IN VARIOUS BRAIN REGIONS
ON AMYGDALOID KINDLING IN RATS AND CATS

Site of Lesion	EFFECT			
	Bilateral or Ipsilateral to Stimulus	Rate of Kindling	Type of Kindled Seizure	Reference
COMMISSURES (Rat and Cat) Anterior only Corpus Callosum and Hippocampal Complete Split Brain		Normal " Faster	Normal Lack Bisymetry " "	4, 4,6,11,10 4,5,10
STRIA TERMINALIS (Rat) STRIA TERMINALIS	Ipsilateral Bilateral	Faster "	Normal "	8, 9,
CUT BETWEEN AMYGDALA AND ENTORHINAL CORTEX (Rat)	Ipsilateral	Faster	Normal	9,
VENTRAL AMYGDALO- FUGAL PATH (Rat)	Ipsilateral	Slower	Normal	9,

TABLE 1 (continued)
THE EFFECT OF LESIONS IN VARIOUS BRAIN REGIONS
ON AMYGDALEOID KINDLING IN RATS AND CATS

Site of Lesion	EFFECT			
	Bilateral or Ipsilateral to Stimulus	Rate of Kindling	Type of Kindled Seizure	Reference
MIDBRAIN RETICULAR FORMATION (Cat)	Ipsilateral	not tested	Blocked Seizures in previously kindled animals	10,13
GLOBUS PALLIDUS	Ipsilateral	not tested	Normal	13,

References

1. Corcoran et al. (1976)
2. McCaughran et al. (1978a)
3. Cain and Corcoran (1978)
4. McCaughran et al. (1978b)
5. Nobrega and Gaito (1978)
6. McIntyre (1976)
7. McCaughran et al. (1976)
8. Engel and Katzman (1977)
9. Racine (1978)
10. Wada and Sato (1975)
11. McCaughran et al (1977)
12. Wada et al (1977)
13. Wada and Sato (1975)

1 - 7 THE TRANSFER EFFECT

It has been observed in rats and cats that, when electrodes are placed into the amygdalae bilaterally and kindling stimulation applied first to one amygdala until the animal is fully kindled and then in the same way to the contralateral electrode, the second amygdala requires fewer stimulations to kindle. This has been called the "transfer effect", first recorded by Goddard et al (1969), but more systematically studied by Racine (1972b) and Burnham (1976), who showed that, following kindling from the amygdala, convulsions were more readily provoked by stimulation of the contralateral amygdala or the anterior cortex, septal region or hippocampus. It was proposed that the emanation of an after-discharge from the primary site was a kindling stimulus which recruited secondary sites in a limbic brain stem seizure circuit.

A "negative transfer" effect was described by McIntyre and Goddard (1973), when they observed that previously kindled amygdala did not generate a full convulsion on the first rekindling stimulation, but sometimes required 4 to 5 stimulations for convulsion following the secondary kindling of the contralateral amygdala. Gaito (1976) and Nobrega and Gaito (1978) extended these observations by showing that the latencies for convulsion differed between homologous sites in a stable way when each amygdala was stimulated alternately for up to 10 trials. It was shown that the transfer effect was unaffected by total forebrain ^{commissurotomy} (McIntyre, 1976) and the fixed difference in seizure latency (described as an "oscillatory effect") was similarly not affected. The conclusion drawn was that both transference and negative transference were dependent on brain stem influences on the amygdala and took the form of excitatory-inhibitory responses to generalized convulsions evoked from the amygdala (McIntyre, 1976 and Nobrega and Gaito, 1978).

However, the experiments which demonstrate the transfer effect have not been designed to control for the effect of electrode implantation in the amygdala for periods of several weeks. In view of the possibility previously referred to, that an electrode itself may cause local changes, which result in an enhanced rate of kindling, all work on the transfer effect and negative effect requires to be reexamined using adequate controls, since it is quite conceivable that the positive transfer effect can be entirely explained by a local electrode effect on one amygdala.

1 - 8 THE EFFECT OF STIMULATING SUBCORTICAL STRUCTURES ON ESTABLISHED KINDLING

Procedures which can cause the arrest or even reversal of established kindling are clearly of major theoretical and practical importance. Much information has been collected on the effects of destructive lesions to various brain structures, but relatively few reports discuss the effects of stimulation. Kovacs and Zoll (1974) reported seizure inhibition in rats by one-hour stimulation of the median raphe nucleus in kindled rats. Tanaka and Naquet (1978) inhibited kindled seizures in cats by stimulating the ventrolateral nucleus of thalamus and central grey matter for up to one hour at 10 Hz. However, the inhibition was short-lasting. Stimulation of the midbrain reticular formation at 300 Hz facilitated kindled seizures. Pinel et al (1973) blocked both the after-discharges and motor seizures in kindled rats by administering foot shock prior to amygdala stimulation, showing that seizure activity could be attenuated by a high level of arousal.

1 - 9 THE PHARMACOLOGY AND BIOCHEMISTRY OF KINDLING

(a) Anticonvulsants

The value of kindling as an experimental model of epilepsy was enhanced by the demonstration that clinically important anticonvulsants, with the possible exception of phenytoin, were extremely effective in delaying or preventing the development of kindling and suppressing or eliminating the ictal features of established kindling. Ashton and Wauquier (1979) tested fifteen clinically useful anticonvulsant agents on rats with established amygdala kindled seizures. All the drugs effectively antagonized the seizures, though with a wide range of potencies. Of added interest was the discovery that cortical kindling and amygdala kindling were affected differently by certain anticonvulsants, including carbamazepine, in a manner which paralleled the differences observed clinically in the effectiveness of drug treatment of temporal lobe epilepsy and idiopathic epilepsy (Albright and Burnham, 1979). Both phenobarbitone and pentobarbitone have a marked prophylactic effect when administered during the development of amygdaloid kindling in rats (Wise and Chinerman, 1974) and cats (Wada et al, 1976) and also have a potent effect in suppressing established amygdaloid kindling seizures in rats (Babington and Wedeking, 1973, Wise and

Chinerman, 1974) and cats (Wada et al, 1976). Both carbamazepine (Wada et al, 1976), which appears to have a more selective effect on the amygdala than the cortex, and valproic acid (Leviel and Naquet, 1977) have a powerful prophylactic effect on kindling in cats.

In contrast, studies using phenytoin in kindling have produced rather inconclusive results. In rats which were fully kindled phenytoin and phenacemide blocked seizures elicited from the amygdala and the cortex, but only at toxic doses which caused ataxia (Babington and Wedeking, 1973). Racine et al (1975) obtained blockage by phenytoin of seizures elicited from the cortex, but not from the amygdala in rats. Wise and Chinerman (1974) found phenytoin had no effect on the rate of development of kindling or the after-discharge threshold in the amygdala of rats. In both rabbits (Tanaka, 1972) and cats (Wada et al, 1976) phenytoin did not suppress kindled seizures. The latter authors delivered daily electrical stimulation to the amygdala at the time of peak plasma level of phenytoin and found the drug devoid of any prophylactic effect.

(b) Antidepressants and Anxiolytic Drugs

Amitriptyline, nortriptyline and imipramine effectively inhibit established kindled seizures and are more potent against amygdaloid than cortically induced seizures. Monoamineoxidase inhibitors were ineffective (Babington and Wedeking, 1973). Diazepam potently blocks both the development of kindling and the expression of seizures in previously kindled rats (Wise and Chinerman, 1974, Babington, 1977). The effect was found in amygdaloid but not cortical kindling (Racine et al, 1975, Racine et al, 1979). Chlordiazepoxide, oxazepam and meprobamate all have similar actions to diazepam (Babington and Wedeking, 1973).

(c) Other Drugs

Other drugs which have been reported to have anti-epileptic properties in kindling include tetrahydrocannabinol (Corcoran et al, 1973 and Corcoran et al, 1978), lidocaine (Tanaka, 1972) and procainehydrochloride (Racine et al, 1975, Racine et al, 1979). The neuroleptics, chlorpromazine and haloperidol, and the antihistamine, chlorpheniramine, were without effect (Babington and Wedeking, 1973).

Drugs which have a stimulatory effect in rats, d-amphetamine and

methyl-phenidate, both prolonged kindled induced seizures elicited from the amygdala or the cortex (Babington and Wedeking, 1973). Similarly, morphine enhanced seizures induced after amygdaloid kindling. This epileptogenic effect was stereospecific and reversed by naloxone (Le Gal La Salle et al, 1977). However, naloxone by itself had no effect on after-discharge threshold or on the rate of development of kindling from the amygdala (Corcoran and Wada, 1979) and this argues strongly against a primary involvement of opiate peptides in kindling.

Babington and Wedeking (1973), who performed very extensive pharmacological studies on kindling, used as a test model rats which had been fully kindled either from the amygdala or the cortex. Drugs were, therefore, tested only for the ability to suppress established seizures. Tricyclic antidepressants have been tested only for this property and, of all agents tested, they have the most potent effect, both on cortical and amygdaloid kindling. Diazepam and the anticonvulsants have been more widely tested in different species and for effects on different aspects of kindling and appear to have powerful prophylactic effects.

(d) Effect of Electroconvulsive Shock on Kindling

In view of the effect of tricyclic antidepressants on kindling, Babington and Wedeking (1975) tested the effect of ECS and showed that electroshock applied to rats 30 minutes, 20 hours or 24 hours prior to amygdaloid stimulation blocked the kindling process. When ECS was discontinued, the kindling process proceeded normally. Like the antidepressant drugs, ECS also suppressed the duration of elicited seizures in amygdaloid kindled rats.

(e) Inhibitors of Protein Synthesis

The biochemical basis of kindling is unknown. However, several processes which are thought to be involved in either growth or plasticity in the nervous system and which must be considered in the context of kindling depend on protein synthesis. Transynaptic induction of enzymes and axonal growth or dendritic sprouting are examples. Furthermore, the critical time interval between stimulations for optimal kindling (about 2 hours) is in keeping with a requirement for a synthetic process. This process must be independent of synaptic activity following the stimulus, since suppression

of neuronal activity by barbiturates immediately after the after-discharge did not hinder the development of kindling (Cain et al, 1979).

The effect of inhibitors of protein synthesis on kindling is, therefore, of great significance. Morrell et al (1976) were the first to demonstrate that inhibition of protein synthesis by cycloheximide in frogs retarded kindling. However, Ogata (1977) challenged the findings by claiming that it was the anticonvulsant effect of the drug and not its protein inhibitory effect which related best with kindling suppression. Recently, following injections to rats of anisomycin in doses which achieved 80% inhibition of forebrain protein synthesis for 90 minutes without causing apparent toxic effect, Jonec and Wasterlain (1979) have reported that kindling is retarded, albeit by a very small amount. The control group kindled after an average of 7.7 stimulations compared to 10.9 for the anisomycin treated group. The effect, it was claimed, was not due to an anticonvulsant effect, since anisomycin injected into fully kindled rats caused no effect on the type or duration of seizure elicited or the seizure threshold. However, as the authors point out, anisomycin has effects other than that of inhibition of protein synthesis and these include inhibition of adrenal steroid synthesis. Rose et al (1979) have recently demonstrated delayed kindling in hypophysectomized rats and a reversal of this kindling deficit by systemic cortisone. It is quite possible, therefore, that anisomycin delays kindling by actions other than inhibition of protein synthesis and steroid suppression is a possible cause. The importance of new protein synthesis during the development of kindling, therefore, remains uncertain.

(f) Acetylcholine and Kindling

The amygdala receives a major cholinergic innervation (Shute and Lewis, 1967) originating in the lateral preoptic area and the ventral fore-brain (Emson et al, 1979), which reaches the amygdala complex via the ventral amygdalofugal pathway (Ben-Ari et al, 1977). It is interesting that Racine (1978) has reported that knife cuts in the ventral amygdalofugal pathway retard amygdaloid kindling and acetylcholine had been proposed to have an excitatory function in kindling (Vosu and Wise, 1975, Arnold et al, 1973).

Effect of Atropine on Kindling

The role of acetylcholine in kindling has been investigated using the muscarinic blocking agent, atropine. In the Sprague-Dawley strain of rats Arnold et al (1973) found that atropine retarded kindling, but Corcoran et al (1976) could not reproduce the findings using hooded rats or cats.

In an attempt to resolve the contradiction in the results from these two laboratories, Albright et al (1979) tested the effect of atropine on kindling in rats using conditions similar to those employed both by Arnold et al (1973) and Corcoran et al (1976). Their results showed a significant effect of atropine in retarding the rate of amygdaloid kindling.

Alterations in Muscarinic Receptors in Kindling

McNamara (1978a) measured a reduction in the numbers of muscarinic receptors in both amygdalae of rats sacrificed 15 hours after the completion of kindling and no change in receptor numbers if rats were sacrificed 3 days after kindling was completed.

Further evidence for a muscarinic cholinergic involvement in the development of kindling, rather than in the long-term changes underlying the maintenance of the process, was provided by observations on interictal spiking in kindling by Fitz and McNamara (1979b).

Spontaneous electrographic spiking occurs in both amygdalae, but is emphasized at the site of stimulus, during the period of daily stimulation leading up to the full development of kindling. Once stimulation is stopped, interictal spiking declines rapidly and is absent by 5 days. The interictal

spike, therefore, seems to be a manifestation of abnormal neuronal excitation in the epileptic focus while kindling is in progress. It was demonstrated that spiking was activated by the muscarinic antagonists, atropine and scopolamine, and reduced by physostigmine and choline chloride; both of the latter treatments increase brain acetylcholine. However, as discussed by Fitz and McNamara (1979b), spontaneous interictal spiking, although reflecting neuronal excitability, probably does not share a common mechanism with kindling and the suppression of interictal spiking is not tantamount to suppressing epileptogenicity. Thus the number of interictal spike drops rapidly when kindling is stopped, whereas the propensity to have seizures in the kindled state is lasting.

Further Evidence for a Cholinergic Involvement in Kindling

Although the effect of atropine on kindling remains controversial, several lines of evidence indicate that, at least in the stages of kindling development, acetylcholine may be influential.

Repeated administration of the cholinergic agonist carbamylcholine using indwelling cannulae placed in the rat amygdala, caudate nucleus or hippocampus (Vosu and Wise, 1975, Wasterlain et al, 1978) resulted in kindling which was similar in all respects to electrical stimulation. Furthermore, when atropine was mixed with the carbamylcholine and both drugs injected the development of kindled seizures was blocked.

Burchfield et al (1978) reported that iontophoretically applied acetylcholine prolonged the enhanced firing rate in hippocampal cells which followed a sequence of kindling stimulations applied to the fornix.

These experiments suggest that acetylcholine may be involved during the development of kindling, but give no support for the view that long-lasting changes in acetylcholine underly the chronic effects of kindling.

(g) Catecholamines and Kindling

The hypothesis that kindling in the amygdala is accompanied by a reduction in the tonic inhibitory action of catecholamines, and of noradrenaline in particular, has been widely backed by both biochemical and pharmacological studies. Furthermore, certain features of the noradrenaline system in the central nervous system suggest mechanisms by which these neuronal systems could support the enduring changes observed in kindling. Most of the nora-

drenaline in the brain originates in the locus coeruleus and other small nuclei in the brain stem and the innervation to the cortex and underlying structures is widespread and diffuse (Lindvall and Bjorklund, 1974). These noradrenergic neurones are able to respond in a lasting manner to alterations in neuronal activity by regulating the new synthesis of the rate limiting enzymes, tyrosine hydroxylase and dopamine β hydroxylase (Thoenen and Otten, 1977).

Pharmacology

The depletion of noradrenaline and dopamine by reserpine and 6-hydroxydopamine (Arnold et al, 1973, Corcoran et al, 1974) and by α -methyltyrosine (Callaghan and Schwark, 1979) facilitates amygdaloid kindling. To distinguish between the effects of dopamine and noradrenaline depletion following the administration of 6-hydroxydopamine, McIntyre et al (1979) and Corcoran and Mason (1979) made use of desmethylinipramine, which protects noradrenergic, but not dopaminergic, neurones from destruction by 6-hydroxydopamine. They found that the rate of kindling was unaltered by the selective loss of dopamine and concluded that the loss of noradrenaline was responsible for the facilitating effect on kindling. It has been further shown that disulfiram, which depletes brain noradrenaline, facilitates kindling, and dopamine agonists, apomorphine and bromocriptine, have no effect on kindling in rats (Callaghan and Schwark, 1979, Farjo, 1978).

The role of noradrenergic β receptors in kindling has been stressed by Callaghan and Schwark (1979), who have shown that the β blocking drug, propranolol, increases the rate of kindling, whereas the α -receptor blocker, phenoxybenzamine, and the dopamine receptor blocker, pimozide, have no influence on kindling.

Tyrosine Hydroxylase

A reduction in tyrosine hydroxylase activity in the stimulated amygdala one month after the last kindled convulsion was shown by Farjo and Blackwood (1978) (Table 2). Tyrosine hydroxylase is a rate limiting enzyme in the biosynthesis of dopamine and noradrenaline (Weiner, 1970, Axelrod, 1971) and a variety of experimental conditions which either enhance (Thoenen and Otten, 1977) or reduce (Reiss and Joh, 1977) the activity of central adrenergic or dopaminergic neurones will lead to a corresponding

TABLE 2

TYROSINE HYDROXYLASE ACTIVITY (nMol ^3H -L-DOPA formed/hr/g wet weight)
IN DIFFERENT BRAIN REGIONS IN AMYGDALOID KINDLED RATS

- each value represents the mean activity \pm S.E. (n)

	Right	Left
<u>Amygdala</u>		
Kindled (7)	1.96 \pm 0.13	#1.31 \pm 0.09*
Sham-operated (7)	2.11 \pm 0.17	#2.10 \pm 0.15
Unoperated (7)	1.95 \pm 0.10	1.98 \pm 0.07
<u>Hippocampus</u>		
Kindled (6)	0.36 \pm 0.02	0.34 \pm 0.04
Sham-operated (6)	0.38 \pm 0.03	0.42 \pm 0.02
Unoperated (6)	0.38 \pm 0.02	0.40 \pm 0.03
<u>Hypothalamus</u>		
Kindled (6)	4.17 \pm 0.28	3.58 \pm 0.28
Sham-operated (6)	4.31 \pm 0.19	4.45 \pm 0.25
Unoperated (6)	5.01 \pm 0.18	4.89 \pm 0.27
<u>Thalamus</u>		
Kindled (6)	1.13 \pm 0.11	0.82 \pm 0.16
Sham-operated (6)	0.89 \pm 0.10	0.88 \pm 0.10
Unoperated (6)	1.10 \pm 0.18	0.92 \pm 0.16...
<u>Striatum</u>		
Kindled (6)	23.99 \pm 1.05	21.79 \pm 0.68
Sham-operated (6)	25.02 \pm 1.63	24.62 \pm 1.24
Unoperated (6)	23.99 \pm 1.66	25.14 \pm 1.85
<u>Cortex</u>		
Kindled (7)	0.28 \pm 0.01	0.26 \pm 0.02
Sham-operated (7)	0.27 \pm 0.02	0.27 \pm 0.02
Unoperated (7)	0.27 \pm 0.02	0.28 \pm 0.02

Site of electrode implantation into the left amygdala

* $P < 0.002$ for comparison of right and left sides (Mann-Whitney-U-test)

change in the activity of tyrosine hydroxylase. The capacity of noradrenergic neurones to adapt to chronic changes in activity by the transynaptic regulation of the synthesis of tyrosine hydroxylase may be of relevance to the neurochemical changes underlying kindling. The reduction in tyrosine hydroxylase activity in kindling could be interpreted to reflect a reduction in the activity of noradrenergic neurones projecting to the amygdala.

Dopamine and Noradrenaline

The endogenous concentrations of dopamine and noradrenaline are poor indicators of the functional activity of catecholamine neurones and various investigations have yielded totally conflicting results after kindling.

In cats, following hippocampal kindling, whole brain dopamine and noradrenaline was depleted bilaterally (Sato and Nakashima, 1976). The turnover rate of dopamine, but not of noradrenaline, was increased as measured in the whole brain hemisphere ipsilateral to stimulus 1 week after the last stimulus. However, the increased dopaminergic activity was probably in striatal and not limbic dopamine. One month after the last kindled convulsion Engel and Sharpless (1977) found decreased dopamine concentration in both amygdalae after kindling and also a reduction in noradrenaline levels, although they considered that the latter could be an effect of electrode placement rather than kindling stimulation. Wilkison and Halpern (1979) reported unchanged basal levels of dopamine and noradrenaline when measured on right and left sides using complete half-brains. In a first report Callaghan and Schwark (1976) reported that noradrenaline was reduced by 50% in the hypothalamus of kindled rats and unchanged in the cortex. In a second report (1979) the same authors recorded a bilateral depletion of noradrenaline in a limbic area which included the amygdala and the hippocampus, and also in the midbrain and frontal cortex. In contrast to their first report, they found normal levels of noradrenaline in the hypothalamus and the discrepancies between their two sets of experiments were not discussed. These authors also reported that dopamine levels were unchanged in all the brain regions studied.

β Receptor

In catecholamine systems, receptor numbers may adapt to the functional activity of neurones. McNamara has used the ligand, dihydroal-

prenolol, to measure noradrenergic β receptors in the amygdala and has demonstrated a reduction in receptor numbers bilaterally three days after a convulsion (McNamara, 1978b).

(h) Other Neurotransmitters

While most attention has been directed to examining the role of dopamine, noradrenaline and acetylcholine in kindling, relatively little has been written about other neurotransmitters.

5-hydroxytryptamine

Manipulation of 5-HT by lesions of the dorsal and median raphe nucleus, which causes a 40% reduction in brain 5-HT, but no change in dopamine or noradrenaline levels, lowered the after-discharge threshold and reduced the number of stimulations required to kindle from the amygdala. There was no significant effect on cortical kindling. However, in contrast, the administration of p-chlorophenylalanine, which inhibits 5-HT synthesis, delayed amygdaloid kindling (Racine and Coscina, 1978). As the authors suggest, these contradictory findings from two procedures which reduce brain 5-HT do not permit a simple hypothesis for the role of 5-HT in kindling.

Gaba and Taurine

Drugs which inhibit gaba-transaminase, γ -acetylenic gaba and γ -vinyl gaba, had a slight anticonvulsant effect on seizures elicited from fully kindled rats (Myslobodsky et al, 1979), but Racine (1978) reported that intraventricular injections of gaba did not influence cortical or amygdala kindled convulsions.

Following kindling of the entorhinal cortex, the release of gaba into a potassium medium from hippocampal slices was enhanced, in rats killed 24 hours after a kindled convulsion (Leibowitz et al, 1978). However, these investigations tell us very little about the function of gaba in the development or the long-term maintenance of kindling.

A single report (Gaito, 1976b) suggested that taurine retarded the development of kindled seizures. However, two other independent studies (Wada et al, 1975 and Burnham et al, 1978) have failed to confirm this. In particular, taurine appears to have no influence on the development of kindling or on seizures evoked in fully kindled rats.

1 - 10 THEORIES ABOUT POSSIBLE MECHANISMS IN KINDLING

Neuronal plasticity may be defined as the capacity of nervous tissue to adapt to changing functional requirements and kindling may be considered in the broader context of plasticity in the adult brain. In no instances are mechanisms of plasticity understood and entirely different mechanisms may underly processes as diverse as memory storage, the adjustment of the range of the auditory systems, the maintenance of circadian rhythms, the neuronal response to injury or the development of an epileptic focus, all of which imply inherently malleable neurones in adult nervous system (Krasne, 1978).

Creager et al (1980) point out that repetitive as well as single pulse activation of synapses in the central nervous system almost invariably influence the magnitude of subsequent responses. In the rat hippocampus they have identified two quite separate types of short-term facilitation unrelated to a third more enduring change in synaptic efficiency described as long-term potentiation by Bliss and Lomo (1973).

However, plastic change observed experimentally in the phenomena of post-activation potentiation and kindling show certain resemblances and it is proposed to discuss how the relationship between input and output in a neuronal system may be modified by use.

The view that use enhances function in nervous tissue is not novel. Descartes in 1649, writing on how we are able to remember objects, proposed the existence of memory traces in the brain. "These traces consist in the fact that the pores of the brain through which the animal spirits have previously flowed because of the presence of that object have thereby acquired a greater facility than the others for being opened again in the same way by the spirits that come to them. And so when they reach these pores the spirits pass through them more easily than through the others..."

More recently, the effect of presynaptic stimulation on synaptic function has been studied in a number of ways, notably by Eccles (1977), who has proposed that permanent plastic changes (including memory) are secured by micro structural changes at certain synapses, developing in response to particular patterns of input stimulus.

The electro-physiological effects of increased neuronal activity were initially investigated using the monosynaptic myotatic reflex in the cat spinal cord. Following the initial observation of Lloyd (1949) that repeated pre-synaptic stimulation enhanced postsynaptic response for a few minutes, Eccles and McIntyre (1951) showed that the duration of the potentiation could be increased to hours in a chronic preparation in which the dorsal root had been cut distal to the ganglion. Root section led to a marked reduction in the reflex discharge in response to a single efferent volley, but following a conditioning train the reflex response was greatly increased. Eccles attributed the initial loss of activity to prolonged disuse (although the effects of neuronal degeneration could not be excluded) and the enhanced response to repeated use during conditioning train.

The generality of the simple view that use enhanced and disuse decreased synaptic function was challenged by Sharpless (1964), who pointed out that it was more commonly observed in the nervous system that use led to a decrease and disuse to an increase in efficiency at the synapse or neuromuscular junction. He cited the phenomenon of habituation, denervation supersensitivity (Cannon and Rosenbleuth, 1949) and the prevention of epileptic change in undercut cortex by the application of repeated electrical stimulation.

Eccles (1977) later also pointed out the inadequacy of a use vs. disuse mechanism of synaptic modification by recognizing that almost all cells in the CNS are discharging impulses continuously and such a mechanism would allow little specificity.

Long-term Potentiation in the Central Nervous System

The monosynaptic spinal reflex had provided a relatively simple circuit for the study of synaptic modulation, but the potentiating effects of stimuli were short-lasting. Enduring potentiation of synaptic transmission following repetitive stimulation was demonstrated in the central nervous system by Bliss and Lomo (1973) and Bliss and Gardner-Medwin (1973b). Exploiting the extreme lamination in the structure of the hippocampus, these authors employed the extracellular recording technique of field potential analysis to study synaptic events in the monosynaptic connection between the perforant path and granule cells in the dendate region of the

hippocampus. In this region fibres of the perforant path which originate in cell bodies in the entorhinal cortex make synaptic connection en passant with apical dendrites of hippocampal granule cells. Following a stimulus applied to the perforant path, extracellular recording electrodes placed in the granule cell layer will record an initial change in potential, thought to reflect synaptic activation in the molecular layer (termed a population excitatory postsynaptic potential). If the population EPSP generated by a perforant path volley are sufficient to activate granule cells, another potential (population spike potential) is generated by the synchronous discharge of many granule cells. In both anaesthetized and active unanaesthetized rabbits the response in the hippocampal granule cell layer was recorded following stimuli applied to the perforant pathway from the entorhinal cortex. Pre-treatment with a conditioning stimulus of 15 per second for 20 seconds caused an enhanced response to a single test volley. The potentiation was long-lasting and was characterized by an increase in the population EPSP, an increase in the spike potential and a reduction of the spike latency. Potentiation seemed to be accompanied by both an increase in the efficiency of synaptic transmission at the perforant path synapse and an increase in excitability of the granule cell population.

The significance of these results lies in the ability of a moderate conditioning regime to cause a long-lasting increase in the efficiency of synapses in the mammalian central nervous system.

The possible relevance of long-term potentiation in the hippocampus to kindling was recognized by Douglas and Goddard (1975), who extended the observations of Bliss and Lomo (1973) to the rat, and showed that potentiation could be produced most reliably by trains of biphasic stimulation at a frequency of 10 Hz or greater, applied to the perforant path only once in 24 hours. They showed that, using this stimulus which was in effect a kindling stimulus, the efficiency of synaptic transmission in the perforant path-granule cell synapse could be enhanced for at least 12 days. When the animals were re-tested 2 months after the last stimulus train the population EPSP remained potentiated, while the population spike had declined to initial levels. It was suggested, to explain these changes that an increase in tonic inhibitory influences on the granule cells had compensated slowly and caused the output of the granule cells to return to normal after 2 months, while the

persisting increase in the EPSP reflected a more stable change in synaptic function.

Further systematic investigation by McNaughton et al (1978) of the increased efficacy of perforant path synapses following high frequency activation has led to the suggestion that the process consists of at least two parts: firstly, a short-term increase in activity, which has the characteristics of post-tetanic potentiation as it occurs in the spinal cord and which persists for up to 10 minutes in the perforant path synapse: secondly, a long-term effect, which was a relatively stable elevation of synaptic response, persisting long after the short-term potentiation had disappeared. These workers demonstrated that long-term potentiation of the perforant path synapse, but not short-term post-tetanic potentiation, was a co-operative process and required the activity of many perforant path fibres. Below a certain critical stimulus intensity, long-term potentiation did not occur, even when post-tetanic potentiation was recorded.

Douglas (1977) reported long-lasting synaptic potentiation in the rat dentate gyrus, using very brief bursts of high-frequency stimulation, which corresponded closely with the normal discharge pattern of the hippocampus, and proposed that long-term potentiation could occur as a normal process in the normal hippocampus. Similarly, Racine et al (1976) have shown that the stimulus most effective in causing kindling is one which mimics the normal firing in amygdala cells. It is an attractive proposition that the process of long-term potentiation in the hippocampal perforant path may have a bearing on the mechanism of kindling. It is proposed that kindling is mediated by a normal adaptive process and is pathological only on account of the abnormal strength of the stimulus, which brings a large number of neurones into enhanced and synchronous activity (Goddard et al, 1978).

Caution, however, is required in the interpretation of results based on extracellular recording of population fields and the observations of Bliss et al and subsequent workers await confirmation by intracellular recording techniques, using hippocampal slice preparations. Nevertheless, long-term potentiation is not limited to the perforant path and has been demonstrated in other hippocampal paths studied, but not in other laminated brain sites such as the cerebellum and olfactory bulb. Unfortunately the technique has not yet

been applied to the amygdala or other regions which kindle readily.

If kindling is due to a process similar to long-term potentiation in the hippocampus, we could expect that certain behavioural functions dependent on limbic plasticity (e.g. learning-memory) might be disrupted after kindling. McIntyre (1979) has presented data to suggest that amygdala kindling is associated with an impaired ability to acquire fear motivated responses.

Kindling, Recruiting and Post-activation Potentiation

Racine (1972b) found that it was necessary to produce an after-discharge for kindling to occur and several weeks of subthreshold stimulation at 60 Hz for 1 second daily did not affect the subsequent rate of kindling in cats. On the other hand, Douglas and Goddard (1975) showed that long-term potentiation of the perforant pathway granule cell synapse in the rat hippocampus could occur without the production of an after-discharge. To test the possibility that any temporary potentiation of synaptic activity, if repeated, may lead to a permanent change in response, Racine et al (1976) compared the characteristics of the potentiated evoked potential following recruiting, tetanic and kindling stimulation.

Responses were measured in the hippocampus after stimuli were applied to the amygdala. Recruitment was measured after 10 Hz train. Post-tetanic potentiation after 50 msec trains of .2 msec pulses at a frequency of 300 Hz applied every 4 sec for a total of 20 trains. An after-discharge was not triggered. The kindling stimulus was for 1 second at a frequency of 60 Hz applied daily with a current just sufficient to evoke an after-discharge.

Following all three types of stimulation the response evoked in the hippocampus was potentiated and the same components were enhanced temporarily by recruiting and tetanic potentiation as were enhanced permanently by kindling. Subsequent kindling was facilitated in rats which previously had received tetanic stimulation, but not recruiting stimulation. Further experiments showed that repeated tetanic stimulation caused a permanent increase of evoked potentials, but did not cause convulsive phenomenon, and the amplitude of the evoked potential was smaller than that produced by kindling. Thus the difference between kindling and PTP could be only quantitative.

These experiments suggested that the stimulus parameters are critical for inducing lasting plastic change. Tetanic stimulation with parameters

which partially mimicked the amygdala after-discharge were most effective in causing potentiation and also for triggering kindled convulsions.

Mechanism of Synaptic Enhancement

Lloyd (1949) proposed that the post-tetanic potentiation he observed in the spinal cord of the cat was related to the presynaptic hyperpolarization which followed repetitive stimulation.

Del Castillo and Katz (1954) supported this view and showed that at the frog neuromuscular junction the size of the end-plate potential is increased by currents which hyperpolarize the nerve endings. Hubbard and Willis (1962) confirmed these findings and further suggested that the rate of transmitter mobilization was increased by the hyperpolarization.

However, Eccles (1977) has suggested that an increase in the presynaptic release of neurotransmitter by itself may not be the basis of a long-lasting modulatory process, since such a system could quickly lead to saturation at maximum efficiency at most synapses.

Bliss and Gardner Medwin (1973) proposed several types of change which would be in keeping with their findings in post-tetanic potentiation and the list, which can equally be applied to kindling, can be extended to include facilitation of synaptic transmission by an increase in the number of synapses: alterations in transmitter release, degradation or re-uptake or change in the number or kinetic properties of receptors. Changes in dendritic morphology and effects on postsynaptic inhibitory inputs can also be invoked. Douglas and Goddard (1975) found no physiological evidence for an increase in the number of presynaptic sites in the perforant path, but thought that both an increase in transmitter release and an increase in postsynaptic sensitivity were involved in post-activation potentiation. The nature of the neurotransmitter involved is unknown, but could be glutamic acid, since Lynch et al (1976) applied glutamic acid by iontophoresis to postsynaptic dendrites in the hippocampus and observed that following post-activation potentiation the increase in cell firing in response to glutamic acid was persistently less than controls. The evidence on whether activation affects only the stimulated synapse, or is a heterosynaptic effect causing a general change in the postsynaptic neurone, has been conflicting, but possibly more than a single process is being observed.

Lynch et al (1977) used a hippocampal slice preparation in which two independent inputs to completely different dendritic subfields of the same pyramidal cells can be monitored, to show that long-term potentiation of one input to the pyramidal cells was accompanied by a persistent depression of the response to the remaining input. They proposed a local potentiation of the driven afferent and generalized depression of the postsynaptic neurone. However, Anderson et al (1977), using a similar preparation, found an increased EPSP specific for the activated input and no change in the response evoked by stimulating the second 'control' input.

It is of interest that Libet (1975) has described in the superior cervical ganglion a heterosynaptic interaction by which one input (dopaminergic) produces a specific and long-lasting enhancement of the subsequent response of the same neurone to another input (which is cholinergic).

Creager et al (1980) reported two processes of short-term facilitation in the hippocampus. The first was limited to the stimulated synapse and the second was a more generalized heterosynaptic response of hippocampal cells to stimulation. However, they stress that both types of short-term change are quite independent of the process of long-term potentiation as originally described by Bliss and Lomo (1973).

Although a number of separate processes may allow limbic structures to respond to use and disuse, only the lasting effect of long-term potentiation bears a close resemblance to the almost permanent features of kindling. However, other modifications of neuronal function may contribute to neurotransmitter related changes observed during the kindling process and immediately following convulsions, and it is essential to consider separately the acute and chronic sequelae of kindling stimulations.

Plasticity and Dendritic Re-Modelling

Eccles (1977) has reviewed circumstantial evidence for the belief that certain modifiable synapses can, in response to use, undergo the processes of hypertrophy, branching and regression, as the basis of long-lasting plastic change in the CNS.

Evidence that such changes do occur is derived mainly from studies on adult brains in the processes of recovery from injury (Cotman, 1978, Azmitia, 1978) and developing brains (Review by Sperry, 1963, Prestige and Willshaw,

1975).

Rats housed in an enriched environment for several weeks are reported to have developed differences in the fine structure of the cerebral cortex when compared with rats from impoverished surroundings. The cortex is thicker and dendrites more branched on pyramidal layers II, IV, V (Watson, 1976). The number of glial cells in the cortex is also increased and although the origin and function of the increase is unknown it is thought to be similar to the glial response which follows electrical stimulation of neurones (Watson, 1972).

Rutledge et al (1974) tested the effect of electrical stimulation on the morphology of dendrites in the cortex. After daily stimulation to the supra sylvian gyrus of cats for several weeks, histological study of the stimulated site and contralateral cortex showed that the apical dendrites were terminally longer and more branched on the contralateral side when compared to a region near the site of stimulus. However, a local effect of the stimulus or stimulating electrode causing loss of dendrites could also explain these findings.

Van Harreveld and Fifkova (1975) specifically tested the suggestion by Bliss and Gardner Medwin (1973) that changes in postsynaptic dendritic spines could contribute to long-term potentiation.

Following tetanic stimulation to the perforant pathway in mice, they observed swelling of the dendritic spines which was present two minutes after the stimulus and had not diminished at 60 minutes. An increase in diameter of the dendritic spine would decrease the length resistance and increase the length constant and thus would facilitate impulse transmission. It was speculated that the uptake of water and electrolytes which causes the spines to swell could be stabilized by a more enduring process, for instance, the formation of structural proteins, to account for the more permanent changes of long-term potentiation. So far this is probably one of the most convincing experiments linking a change in dendritic morphology with a specific change in function.

Biochemistry of Synaptic Plasticity

Biochemical changes which accompany prolonged synaptic stimulation have mainly been studied in peripheral nerves (Review by Giacobini, 1970,

Thoenen, 1975). Thoenen has pointed out that the response of a neurone to a depolarizing stimulus is not confined to immediate changes such as membrane permeability to ions, but includes effects on the regulation of synthesis of cell constituents, and these slower mechanisms may be involved in long-term adaptation. One process which has been particularly well studied is the induction of tyrosine hydroxylase and dopamine B hydroxylase in peripheral adrenergic neurones in response to prolonged increase in the activity of adrenergic neurones. In central adrenergic neurones it has been less intensively studied, but the induction of TOH has been reported following electroshock, long-term reserpine treatment and environmental isolation. Thoenen (1975) reviews the evidence that the transynaptic induction of TOH is a specific response to increased cholinergic stimulation of adrenergic neurones and not part of a general increase in protein synthesis. Regulation is at the level of transcription and increased amounts of enzyme are synthesized in the perikarion and subsequently transported to the nerve terminal, where increased synthesis of ^{NORADRENALINE} \ provides a lasting form of adaptation.

Chubb (1975) reviewed the evidence that long-term adaptation can be effected by exchange between neurones of substances which cannot be considered as neurotransmitters. Secretion products of sympathetic and motor nerves include proteins and other compounds which appear to be involved in the normal growth, development and maintenance of innervated tissue. Schubert and Kreutzberg (1974) demonstrated the transfer of adenosine and uridine derivatives from the cell body of a neurone to intracellular sites in the postsynaptic cell. Furthermore, this transfer was enhanced by stimulation of the presynaptic cell (Schubert et al, 1976). McIlwaine (1977) has used these findings to propose "second messengers" which, transported by cytoplasmic flow, are concerned with events after the release and inactivation of transmitter at the synapse, and which can modify cell firing rates for long time periods after neuronal stimulation.

Conclusion

Neuronal function can be modified either transiently or permanently by a variety of patterns of applied electrical stimulation and a number of biochemical and morphological processes may underlie these changes, which may provide a clue to the mechanisms of kindling.

However, a further consideration is that, apart from having synapses which are inherently capable of plastic change, the nervous system needs ways to regulate plastic modifications when these would be maladaptive. It is, therefore, possible that kindling, rather than having a direct influence on potentially plastic synapses themselves, works by interfering with systems which have a permissive or inhibitory influence in the elaboration of plastic change (Krasne, 1978).

However, investigations of such regulating systems are beyond the scope of our present knowledge of plasticity.

The hypothesis that during amygdaloid kindling catecholamine systems are inhibitory and cholinergic systems excitatory stemmed from the early observations that treatment with reserpine or 6-hydroxydopamine enhanced the rate of kindling, whereas atropine was thought to have a suppressing effect (Arnold et al, 1973). My first aim was to test this hypothesis by the biochemical experiments which are introduced and described in chapter 2.

Dopamine and noradrenaline were measured in the amygdala and the hippocampus and the turnover rates of these amines calculated by measuring their rate of depletion following α -methylparatyrosine injection.

In view of the proposed influence of cyclic GMP on neuronal excitability and the reported relationship between the cyclic nucleotide and catecholamines, cyclic GMP was assayed in slices of the amygdala of kindled rats.

Cholinergic function was assessed in kindling first by a re-examination of the effect of atropine on the development of kindling, using two different dosages of the drug. Muscarinic receptor numbers were measured using a radioligand binding assay and high affinity sodium dependent choline uptake, a parameter which closely reflects cholinergic neuronal activity, was assayed. Acetylcholinesterase, a membrane bound enzyme, was also measured.

To test whether impaired ionic pump mechanisms could play a part in kindling, ATPases were measured. These enzymes, which are membrane bound, could also be considered as markers for membrane performance and for the detection of glial cell damage caused by electrode implantation.

During the experiments on the effect of atropine in the development of kindling it became apparent that the prolonged presence of an electrode in the amygdala without stimulation could reduce the number of stimulations required for subsequent kindling. This proposition was examined using both stainless steel and platinum/iridium electrodes in experiments which are described in chapter 3.

Finally, a relative measure of regional glucose utilization was obtained using ^3H -deoxyglucose in order that neurotransmitter related changes could be compared with and contrasted to alterations in metabolic activity. Experiments employing deoxyglucose are introduced and discussed in chapter 4.

CHAPTER 2

Ch. 2 THE BIOCHEMISTRY OF KINDLING

2 - 1 INTRODUCTION

(a) Turnover of Dopamine and Noradrenaline

The introduction of effective inhibitors of catecholamine biosynthesis provided a means of estimating catecholamine turnover in brain. Changes in the turnover rate of monoamines may be a better guide to functional activity than changes in amine concentration.

Brodie et al (1966) exploited the properties of α -methyltyrosine, a reversible inhibitor of tyrosine hydroxylase, to measure the turnover rates of dopamine and noradrenaline. The amine concentrations decline at an exponential rate after blockade of synthesis, obeying first order kinetics, since in a steady state synthesis of transmitter balances removal and the removal is proportional to concentration.

Iversen and Glowinski (1966) compared the use of α -methyltyrosine for measuring the rate of turnover of noradrenaline in various regions of the rat brain with two other methods and found no significant differences in the estimates obtained. The other methods were:

1. The rate of disappearance of labelled noradrenaline after the intraventricular injection of ^3H dopamine.
2. The rate of disappearance of ^3H noradrenaline after the intraventricular injection of DL - ^3H noradrenaline.

I, therefore, used the α -methyltyrosine procedure and measured the basal levels of dopamine and noradrenaline and their turnover rate and turnover time in the right and left amygdala and hippocampus of kindled, sham-operated and unoperated controls.

(b) Cyclic GMP in Kindling

Cyclic guanosine 3', 5' monophosphate (cyclic GMP) has been proposed as a regulator of neuronal excitability (Ferrendelli, 1976) and changes in cyclic GMP concentration have been reported in experimentally produced epilepsy. In the mouse cerebellum, cyclic GMP levels increased following electroconvulsive shock or treatment with convulsants and were reduced after treatment with central nervous system depressants and anti-convulsant

agents (Lust et al, 1976, Lust et al, 1977). Elevated levels of cyclic GMP were measured during penicillin-induced focal epilepsy in cat cerebral cortex (Raabe et al, 1978) and pentylene-tetrazol induced seizures in mice (Ferrendelli et al, 1976a).

The long-lasting nature of the changes in kindling permits the underlying alterations in neuronal excitability to be studied independently of the immediate effects of the seizures themselves and this study was designed to show whether the long-term changes of kindling are associated with changes in the basal level of cyclic GMP and in the response of cyclic GMP to Ca^{++} and depolarizing concentrations of K^{+} in brain slices. In view of the claims that changes have been observed in catecholamines in the amygdala after kindling, I also measured cyclic GMP concentration in amygdala slices incubated in buffer containing dopamine and haloperidol. To my knowledge there are no reports of the response of cyclic GMP to drugs or putative neurotransmitters in the rat amygdala nuclei, but it is of interest that a relationship between cyclic GMP and dopamine has been observed in the rat striatum, where a reduction in dopamine caused by a 6-hydroxydopamine lesion in the substantia nigra is accompanied by an increase in striatal cyclic GMP (Carenzi et al, 1976). In the mouse cerebellum dopamine depresses and noradrenaline elevates cyclic GMP (Ferrendelli, 1976) and in cortical slices from the rat neuroleptic agents, including haloperidol, have the effect of elevating cyclic GMP concentrations (Palmer et al, 1976).

(c) Experiments to Assess the Role of Acetylcholine in Kindling from the Amygdala

The effect of atropine on kindling

In view of the conflicting results which had been published on the effect of atropine on the rate of development of kindling in rats, I examined the rate of kindling and the after-discharge threshold in groups of rats which had been treated with atropine in a dose of 25 mg/kg and 2.5 mg/kg and compared the results with saline-treated controls.

The effect of drugs on the development of kindling may not provide any insight into the type of neuronal process which makes kindling stable for several months or longer, although it may tell us something about the establishment of the phenomenon.

Muscarinic receptors

Cholinergic function in the amygdala was examined one month after the last kindled convulsion by measuring muscarinic receptor numbers and sodium-dependent high affinity choline uptake in the amygdala.

A relationship between the number of muscarinic receptors and cholinergic activity in the cholinergic pathways of the central nervous system has not been clearly established. However, in the nigro-striatal system, when dopaminergic neurones are destroyed unilaterally by 6-hydroxydopamine, thus enhancing the activity of striatal cholinergic interneurones by the loss of inhibitory dopaminergic input, specific ^3H -atropine binding was reduced on the side of the lesion and increased on the contralateral side (Kato et al, 1978). These findings have been independently corroborated using QNB (L. Smart unpublished) and support the view that in some circumstances following increased cholinergic activity there is a compensating reduction in muscarinic sites on cholinceptive neurones. A similar process is observed in the noradrenergic system, since the number of ^3H -isoproterenol binding sites is increased in rat cerebral cortex following destruction of the ascending noradrenergic neurones (Sporn et al, 1976).

Choline uptake in kindling

The high affinity choline uptake system is the rate limiting and regulating step in acetylcholine synthesis at the nerve terminal and this transport process is coupled to neuronal function, thus providing a sensitive measure of the functional activity of cholinergic neurones (Kuhar and Murrin, 1978).

Choline uptake has not been previously measured during any of the stages of kindling and I assayed various regions of rat brain for choline uptake activity one month after the completion of kindling to investigate the role of acetylcholine in kindling maintenance.

Klemm and Kuhar (1979) have shown that the activity-related changes in choline uptake, such as the increased hippocampal uptake following pentylenetetrazol treatment or the reduced uptake following barbiturate treatment, is subject to rapid post-mortem reversal. To ensure that my assay technique was sensitive enough to measure changes in choline uptake which reflected changes in cholinergic activity in vivo, I also assayed choline

uptake in the hippocampus of rats treated with barbiturate.

Acetylcholinesterase activity following kindling

The activity of this membrane-bound enzyme does not accurately reflect
CHOLINERGIC ACTIVITY

We measured this enzyme as ATPase was measured, partly to exclude extensive tissue damage at the site of stimulus in kindling.

(d) ATPase and Kindling

ATPase was measured in kindling, both as a marker for possible tissue reaction and because changes in ATPase activity have been implicated in the epileptic process in both animal and human tissue.

To interpret the significance of neurochemical changes at the site of stimulation in kindling, it is essential to establish that changes are not occurring as a result of generalized tissue damage. In response to platinum electrode implantation in the hippocampus, Robinson et al (1975) have demonstrated changes in glial oxidative enzyme activities and also in total ATPase in a narrow region round the electrode. These changes persisted for up to 60 days and were not secondary to electrical stimulation.

One possible site for a lesion in epilepsy is the membrane pump responsible for maintaining the potential difference across a conducting membrane. Tower (1969) has demonstrated an impairment in the ability of cortical tissue taken at operation from epileptic patients to re-accumulate potassium and Rapport et al (1975) found a reduction in Na^+/K^+ ATPase in human epileptic cortex, although he could not rule out the possibility that these changes were merely secondary to the gliosis which was present at the epileptic focus. Furthermore, ouabain - a specific inhibitor of pump Na/K ATPase - is a powerful convulsant (Donaldson et al, 1971 and 1972).

ATPase not involved in pump activity may also be implicated in epilepsy. Rosenblatt et al (1976 and 1977) have proposed that in some strains of seizure-prone mice the tendency to convulse at particular stages in development may be caused by a defect in the synthesis of a membrane Ca^{++} -ATPase which is Ca^{++} dependent, and which is situated on the plasma membrane of glial cells (Trams and Lauter, 1978).

2 - 2 METHODS

Regular Care of Animals

Male albino Wistar rats used in all experiments were housed not more than four to a cage and fed Oxoid 41 diet available ad libitum and water ad libitum. For 1 week after operation the diet was supplemented with brown bread and milk. During kindling animals were handled at least once daily and at all other times the animals were handled at least three times weekly. The animal house was maintained at a temperature of $70 \pm 2^{\circ}\text{F}$ with a 12-hour dark-light cycle 8 a.m. - 8 p.m. The operative procedures and electrical stimulations did not normally cause any evident stress and in any instances when the post-operative recovery was complicated by scalp wound infection, neurological deficit or overt stress those animals were sacrificed.

Construction of Electrodes

Recording screw electrodes

Hollow stainless steel screw electrodes (8 BA) about 8 mm. in height were made in the department. The diameter of the screw allowed the electrode to fit securely into a craniotomy made by No.6 burr size, and the hollow centre made tight connection with a male 'centiloc' connector.

Stimulating/recording electrode implanted in the amygdala

These were made using either triple teflon coated 36 guage stainless steel wire from Phoenix Wire Inc., Vermont, or from 5/1000 inch wire made of 90% platinum and 10% iridium coated with 1/1000 teflon (obtained from Clark Electromedical Instruments, Reading).

Two 1 cm. strands of wire were twisted together. At one end the teflon coating was scraped from the terminal 1 mm. portion and the wires crimped into female gold-plated 'centiloc' connectors (CTA-CP and CTA-CS). The centiloc connectors were held approximately 1 mm. apart in insulating strip and the assembly was secured by 'Simplex' acrylic cement (Howmedica International Ltd., London).

Centiloc connectors and insulating strip were bought from ITT Cannon Electric, Los Angeles.

The 'female' centiloc connectors projected from the top of the rat's head and connection with the stimulating and recording apparatus was effected

by 'male' gold'plated connectors crimped on to the stimulating leads made from 10/0.1 miniature stranded wire, and held fast by heat shrinkable sleeving from R.S. Components, London.

Before use the resistance of all electrodes was tested and those with a short circuit were discarded.

The Operation for the Implantation of Electrodes

Rats weighing 180 - 200 g. were used. Anaesthesia was induced by the inhalation of air saturated with halothane ("Fluothane", I.C.I.) and maintained at a surgical level throughout operation by the inhalation of an air-halothane mixture delivered from a standard anaesthetic machine (British Oxygen) through a specially constructed face nozzle.

The co-ordinates for the placement of the electrodes were derived from the stereotaxic atlas of the rat brain by Konig and Klippel (1963). Accordingly, rats were secured to a stereotaxic frame (David Kopf) by ear bars which delineated the frontal zero plane, and an upper incision bar which was adjusted 2.4 mm. below the interaural line.

A longitudinal incision was made in the skin over the vault of the cranium and a skin flap turned to expose the skull. After haemostasis was achieved, two burr holes were made using a dental drill and No.6 burr size, in the frontal bone 2 mm. anterior to the coronal suture and 2 mm. lateral to the sagittal suture on both sites. Stainless steel recording electrodes were secured into these holes and fixed in place by dental acrylic cement. (Figure 2).

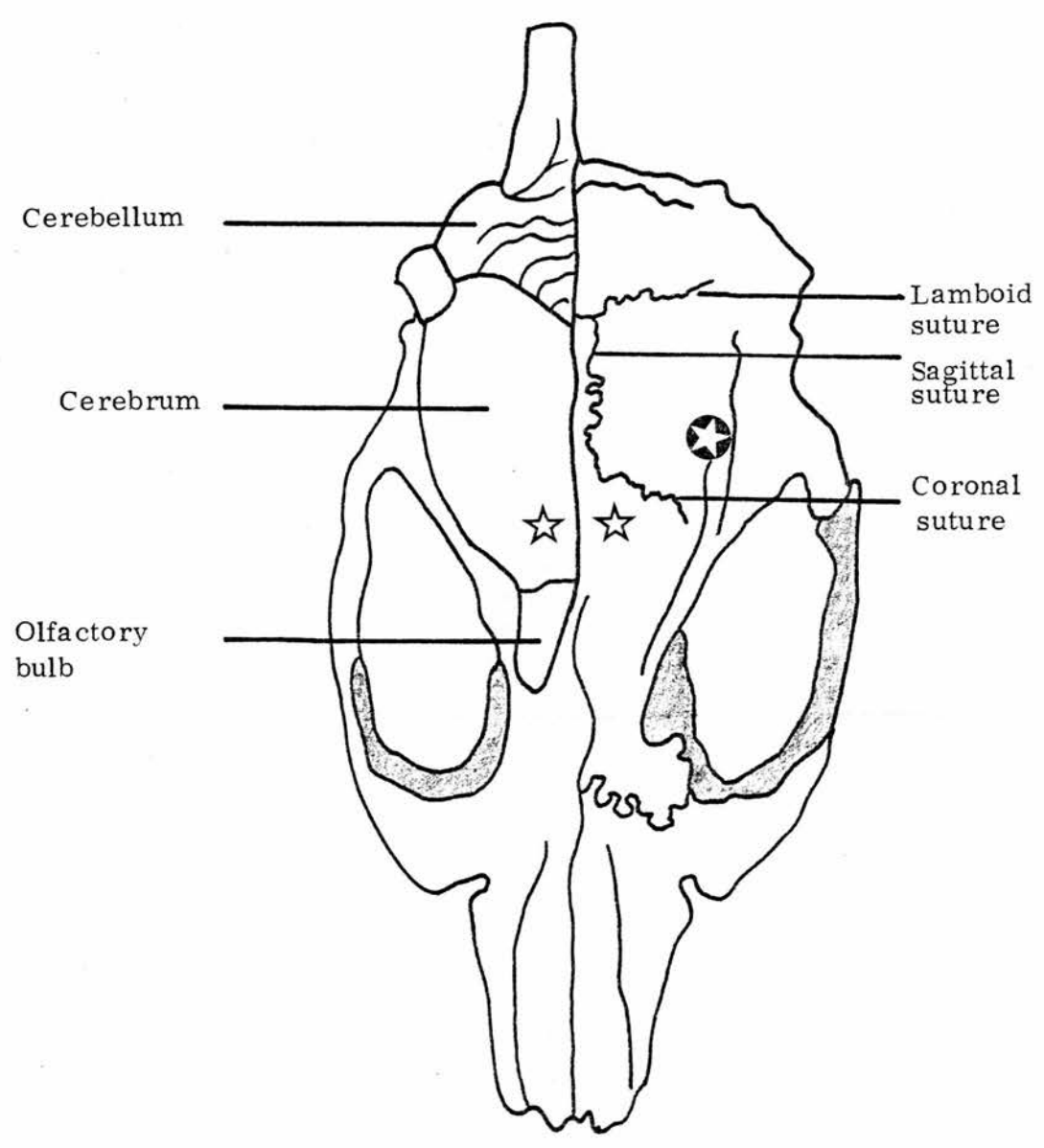
A third craniotomy on the left 4.5 mm. anterior to the interaural line, 4.4 mm. lateral to the midline was made by a No.6 burr. The dura was observed with the aid of an operating microscope (Zeiss) and carefully penetrated with a scalpel tip. The bipolar electrode was then advanced through the brain substance until its tip was 7.9 mm. below the level of the dura. Bleeding was occasionally moderate if a subarachnoid vessel had been ruptured, and, only when the operative field was dry, acrylic cement was applied to fix the electrodes to the cranium. The skin flap was re-sutured with silk.

Post-operative Care

Operated animals were housed together in groups of no more than 4 and allowed 6 or 7 days for recovery before stimulus was applied to

FIGURE 2

SITES OF ELECTRODE INSERTIONS FOR KINDLING



Implantation site of amygdala electrode



Implantation site of cortical screw electrodes

DORSAL ASPECT OF RAT SKULL ONE HALF OF BRAIN
SURFACE EXPOSED

commence kindling.

Stimulation Parameters

In all kindling experiments the stimulus was a 1 second train of monophasic square wave pulses at frequency of 60 Hz. The current was set for each animal above the threshold to evoke an after-discharge and was in the range 50 μ a - 400 μ a. In most experiments a current of 300 μ a was used for all animals, as this was suprathreshold in all rats which had correctly placed electrodes.

The stimulus was generated by a Farnell constant voltage generator, connected to a Neurolog NL 800 system which converted the output to constant current. The stimulus current was monitored on an ammeter which was connected to the circuit via a 3-way switch. The bipolar electrodes in the left amygdala were used both for delivering the stimulus and for recording electrical activity which was amplified and recorded on a Grass polygraph (model 7) before and after stimulus delivery. The recording paper speed was 10 mm/sec. and the duration of an after-discharge was measured in seconds from the end of the stimulation (Figure 3).

Kindling Development

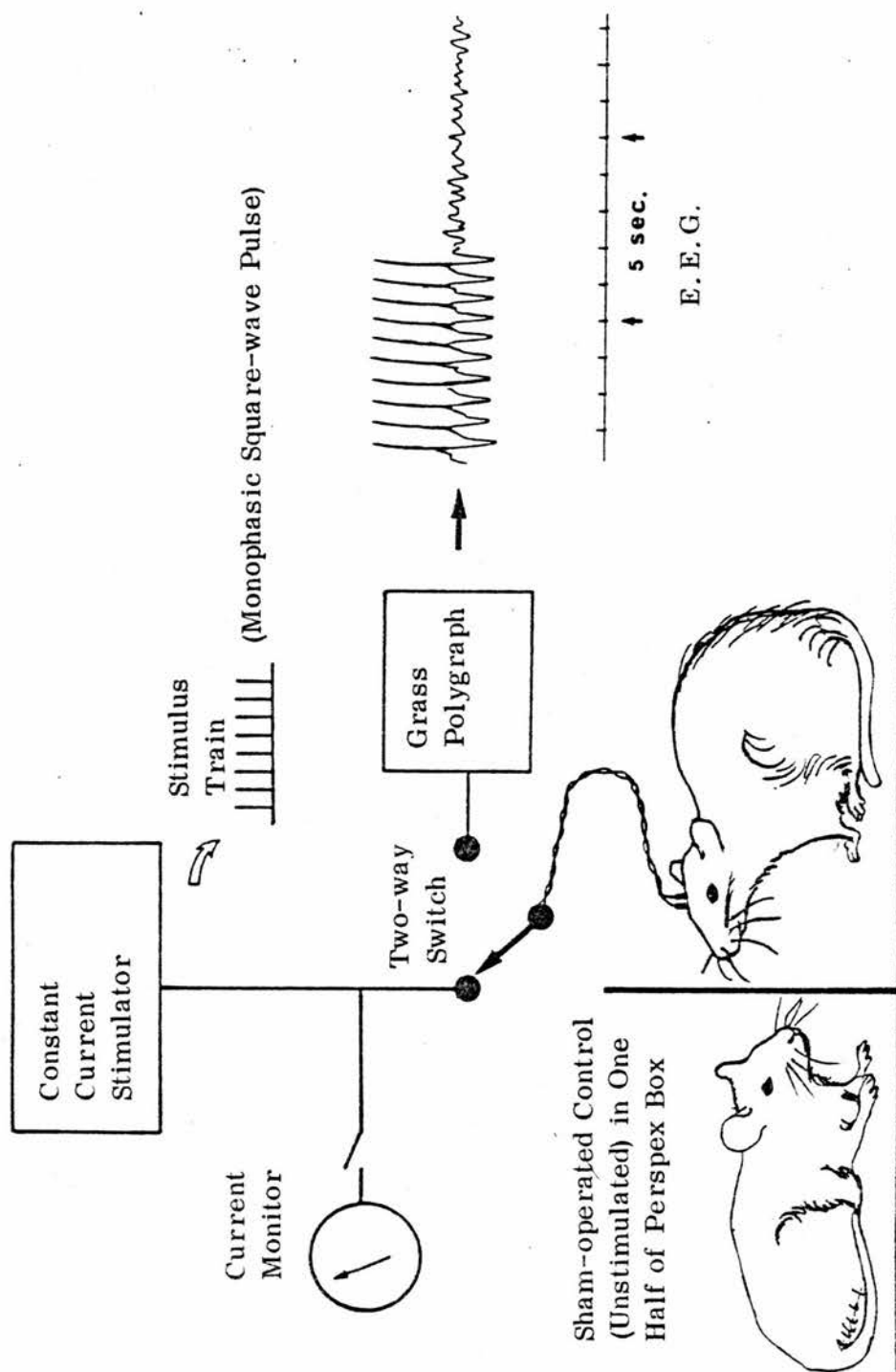
Animals were stimulated either once every 24 hours between 9 a.m. and 1 p.m. or twice daily between 9 a.m and 6 p.m., when at least 4 hours elapsed between stimulations. Stimulation was usually done on weekdays only, but in some experiments daily stimulation was performed. At each stimulation the stimulus intensity, the length of the after-discharge and the behavioural response of the animal were recorded. These were classified as:

<u>Stage 0</u>	Movement arrest or sometimes increased activity, but no seizure phenomena.
<u>Stage 1, 2</u>	Mouth and jaw movements. Repetitive nodding, closure of eye ipsilateral to stimulus.
<u>Stage 3</u>	Forelimb clonus.
<u>Stage 4</u>	Rearing on hind legs.
<u>Stage 5</u>	Rearing and falling backwards. Hypersalivation.

A distinction between Stages 1 and 2 as described by Racine et al (1972) was found to be unreliable, as the response was very variable. In contrast, the development of Stage 3 (forelimb clonus) appeared to be the most frequent,

FIGURE 3

DIAGRAM OF THE KINDLING PROCEDURE



easily observed and unambiguous sign that the fit was developing into a generalized seizure.

Rats were considered fully kindled when a stimulus evoked a Stage 5 response on 3 consecutive occasions. Kindled rats were housed in groups of 2 - 4 and regularly handled until sacrifice, without further stimulation.

Typical electrographic recordings are shown in Figures 4 and 5.

The Dissection of the Brain

The following dissection procedure was used to obtain tissue for the assay of ATPase, acetylcholine esterase, muscarinic reception numbers, noradrenaline, dopamine and cyclic GMP. The dissection used in experiments involving deoxyglucose is described in chapter 4.

Rats were removed to the laboratory and to minimize the effects of stress were allowed at least half an hour of warmth and quiet before sacrifice by a blow on the thorax and decapitation by shears. The skull was opened and the whole brain removed and rapidly cooled to between 4 and 10°C in a beaker of physiological saline left on ice. Normally the brain reached the saline well within 60 seconds of death.

Dissection was done on a wad of filter paper kept at about 0 - 5°C in a Lager cooler. A full thickness sliver of cortex was sliced from both sides using a scalpel blade. The hippocampus was exposed by cutting through the cortex and the corpus callosum from above and the entire structure was peeled aside after sectioning the fornix. A remnant of the inferior part of the hippocampus, which lies posteriorly in close proximity to the amygdala, was not removed, but the dissection included the main parts of both the dentate gyrus and the hippocampus proper.

The brain was then turned over so that the ventral aspect became uppermost and a vertical section through the frontal plane was made at the level of the chiasma (A 5700 μ), using two razor blades.

The corpus striatum (caudate/putamen and the globus pallidus), readily identified by its striate appearance, was gouged out, using scissors and fine forceps, from the anterior brain section formed by the cut. No attempt was made to dissect the small parts of the striatum which remained posterior to the level of the cut.

FIGURE 4

EEG RECORDED IN THE EARLY STAGES OF KINDLING

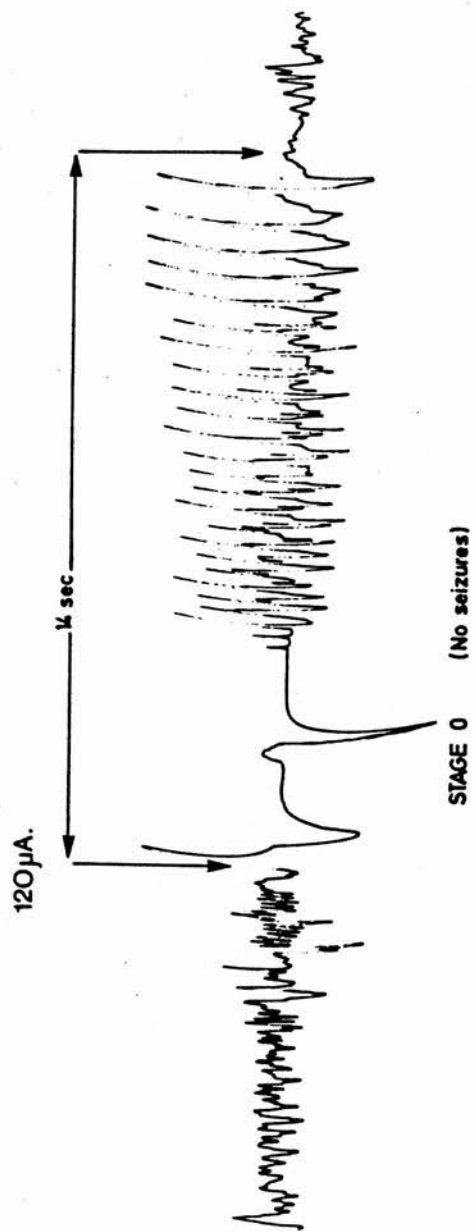
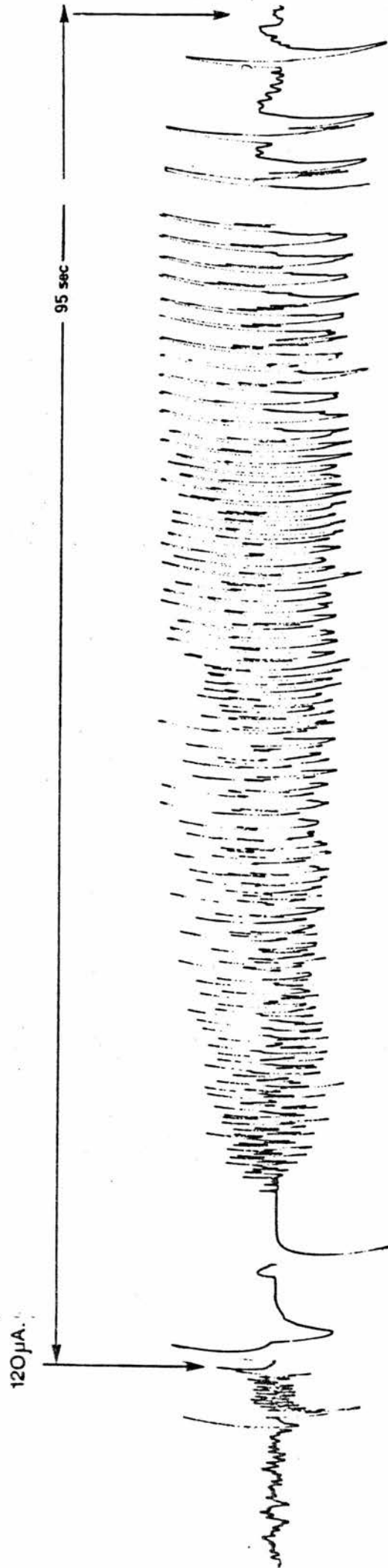


FIGURE 5

EEG RECORDED DURING A GENERALIZED SEIZURE INDUCED BY KINDLING



STAGE 5 (Generalised convulsion)

Note: Compared with EEG recorded in early stages of kindling this trace has:

1. Longer duration
2. Faster frequency
3. More complex wave form.



52.

A second vertical knife cut through the frontal plane was made at the level where the crus cerebri meets the pons (A 3000 μ). The amygdala and the hypothalamus were dissected, using fine scissors and a sharp pointed scalpel blade, from the middle brain section (i.e. between A 5700 μ and A 3000 μ), as illustrated in Figures 6 and 7.

With the amygdala were included parts of the pyriform cortex, the commissural part of the stria terminalis and the lateral olfactory tract.

The midbrain reticular formation was dissected from the posterior brain section by making vertical cuts through the medulla oblongata and using landmarks illustrated in the rat atlas of Konig and Klippel to aid identification.

The reproducibility of the dissection is shown in Table 3, which gives the standard deviation from the mean of the wet weights obtained from 108 consecutive dissections of amygdala, striatum and hippocampus over a two-year period. Only a small number of hypothalamic and reticular formation dissections were carried out and the mean weights and standard deviations of these are also listed.

Measurement of Noradrenaline and Dopamine Concentration and Turnover

Animals were randomly allocated prospectively to the sham-operated, kindling or unoperated groups. Kindling was performed in the usual way and animals in the kindling group which had faulty electrodes or which did not kindle characteristically were discarded. Sham-operated controls were placed in one section of the perspex kindling box, at the same time as a kindling rat was in the second section. Thus sham and kindling rats received the same treatment in all respects except for receipt of an electrical stimulus. Unoperated controls were age matched and handled regularly. One month after the last kindled convulsion of rats in the kindled group, all animals were killed and their brains dissected in the usual way. Tissue samples were weighed and transferred to polycarbonate tubes for storage in liquid nitrogen (-196°C) until required for assay of dopamine and noradrenaline.

The Assay of Dopamine and Noradrenaline

The method was that of Coyle and Henry (1973) and Palkovits et al (1974). The method is based on the methylation of catecholamines to 3-methoxycatechols by rat liver catechol-o-methyltransferase, the oxidation of the methy-

FIGURE 6

PARASAGITTAL SECTION OF BRAIN OF RAT ABOUT 2.5 mm TO ONE SIDE OF

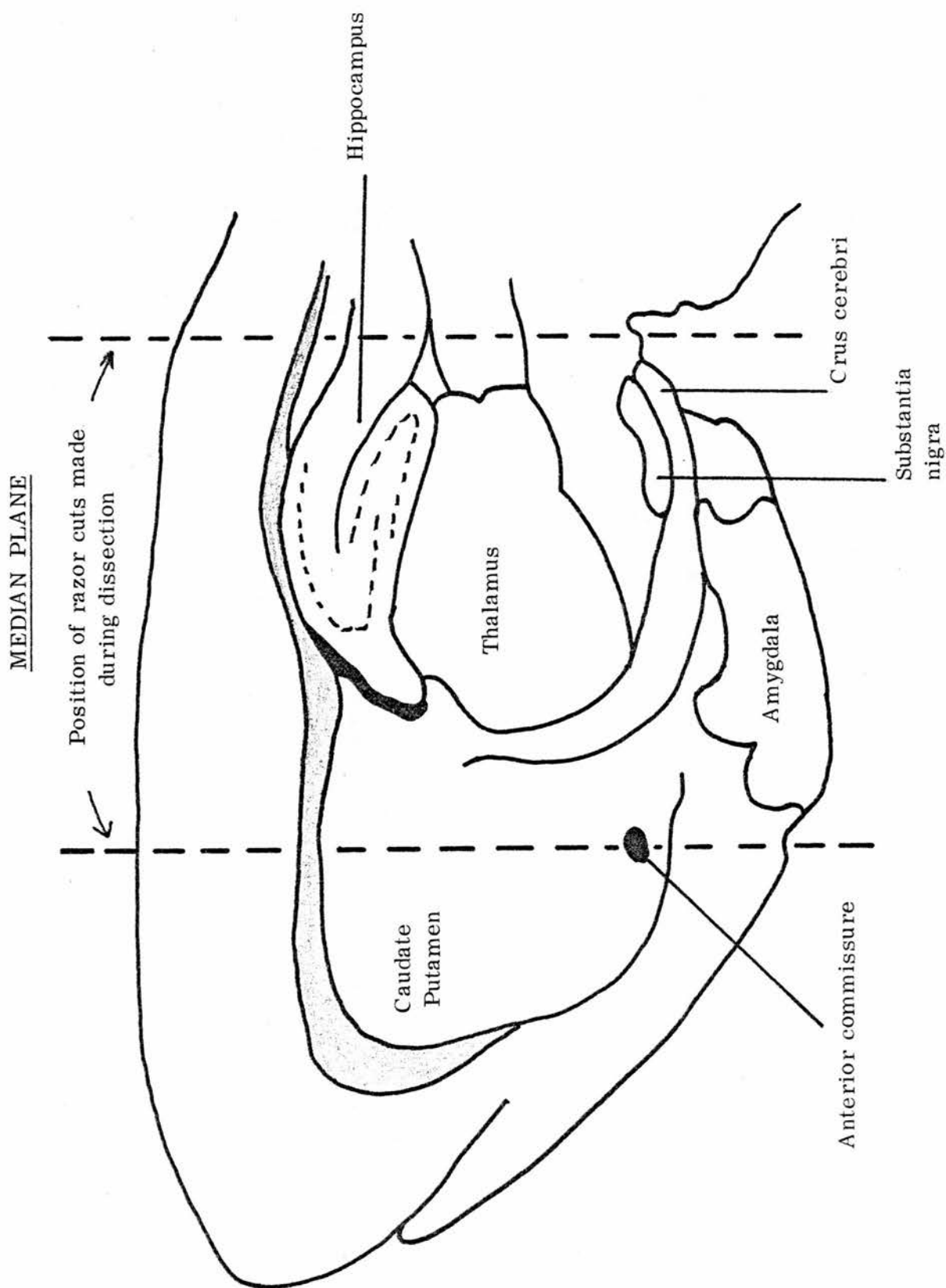


FIGURE 7

SECTION THROUGH FRONTAL PLANE OF BRAIN OF RAT ABOUT 0.5 mm POSTERIOR

TO THE CHIASMA

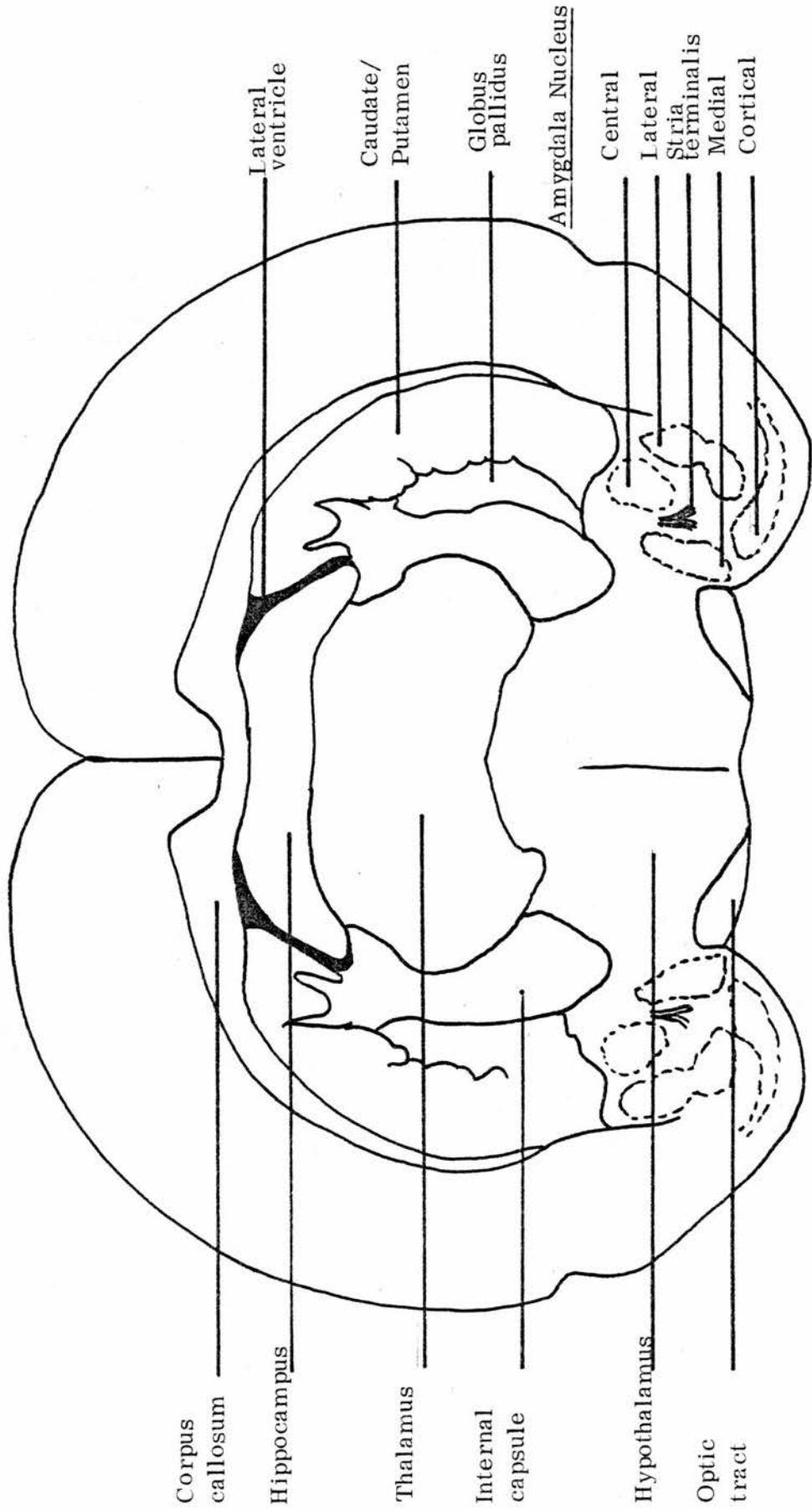


TABLE 3REPRODUCEABILITY OF DISSECTION

The wet weight (mg) of brain regions dissected bilaterally from a total of 108 consecutive rats over a period of two years.

<u>Brain region dissected</u>	<u>Mean wet weight (mg)</u>	<u>S. D.</u>	<u>N.</u>
Amygdala	20.2 mg	4.0	216
Hippocampus	41.5 mg	8.1	190
Striatum	34.6 mg	6.4	216
Reticular formation	33.9 mg	13.3	38
Hypothalamus	51.9 mg	13.7	38

lated products and the separation of the derivatives of dopamine and noradrenaline by a series of extractions. The β -hydroxylated catecholamines, noradrenaline and adrenaline, cannot be distinguished from one another by this method.

Preparation of homogenate

After removal from storage in liquid nitrogen, the polycarbonate tubes containing brain samples were placed on dry ice (-76°C) until homogenization, which was performed in ice-cold 0.1 M-perchloric acid (30 μl PCA to 1 mg. wet weight), using a glass homogenizer at a speed of 1000 - 2000 rpm for 30 seconds. The procedure was adequate to disrupt cells and a nuclei and debris free homogenate was obtained after centrifugation at 1000 g for 15 minutes at 0°C (MSE Mistral 2L).

Samples of the supernatant were used for the assay, which was performed in duplicate on all specimens.

Methylation of catecholamines

To 300 μl of the supernatant in 15 ml conical glass centrifuge tubes was added 100 μl of a freshly prepared solution containing:

500 μg Dithiothreitol (Cleland's reagent. Sigma)
 10 μl 0.5 μMoles MgCl_2
 40 μl 140 μMoles Tris buffer pH 9.6
 25 μl *CONT* solution
 25 μl ^3H -sulphur-adenosyl-methionine (2.5 μCi in 0.17 nmoles)

Dithiothreitol was found to be unstable and care was exercised to use only fresh preparations, which were weighed with a plastic spatula to avoid metal contact.

The mixture was incubated at 37°C in a water bath for 60 minutes and the reaction was stopped by plunging the tubes into ice and adding 500 μl of 0.5 M-borate buffer pH 10.

50 μl of freshly prepared non-radioactive carrier was added, containing:

7 μg Methoxytyramine (Sigma)
 3 μg Normetanephrine (3-Methylnoradrenaline) (Sigma)
 3 μg Metanephrine (3-Methyladrenaline) (Sigma)
 1 μg EDTA

O-methylated products were extracted into 9 ml. of water saturated ethylacetate-methanol mixture (10:1) by shaking for 30 seconds. Phase separation was completed by centrifugation at 0°C for 5 minutes at 1000 rpm (Mistral 2L centrifuge). 8.5 ml. of the ethylacetate phase was transferred to another tube and washed with 0.5 ml. of 0.5 M-borate buffer (pH 10). The tube was shaken for 30 seconds and centrifuged at 1000 rpm. 8 ml. of the organic phase was shaken vigorously for 30 seconds with 0.5 ml. 0.1 M-HCl and centrifuged at low speed. The organic phase was aspirated off and discarded. The acid phase was washed with 8 ml. of water saturated ethylacetate and the organic phase discarded. The glass tubes were transferred to an ice bath and 0.5 ml. of 0.5 M-sodium phosphate buffer pH 7.5 was added to each tube.

The separation of the methylated products of dopamine and noradrenaline

50 μ l of freshly prepared 3% (w/v) sodium metaperiodate was added to each tube and after exactly 3 minutes the oxidation was stopped by the addition of 50 μ l 10% (v/v) glycerol. 3-methyl noradrenaline is thus converted to vanillin by cleavage of the side chain at the β hydroxyl group, whilst 3-methyl dopamine is unaffected.

(3 H-Methyl) vanillin was extracted into 10 ml. of toluene and the aqueous phase was set aside for dopamine determination.

Estimation of noradrenaline

9 ml. of the toluene phase was transferred to a tube containing 1 ml. 1M-NaOH. Vanillin partitioned into the aqueous phase and the organic phase was discarded. The aqueous phase was acidified with 0.1 ml. glacial acetic acid and the vanillin was again extracted into 10 ml. toluene. 9 ml. of the organic phase were transferred to a counting vial containing 400 μ l Liquifluor (New England Nuclear) and counted for tritium for 10 minutes in a liquid scintillation counter at an efficiency of 36%. ('Liquifluor' is a concentrate of PPO and POPOP in toluene).

Estimation of dopamine

To the aqueous phase following the periodate cleavage reaction 5 ml. toluene was added, shaken, centrifuged, aspirated and discarded. 0.5 ml. of 1M-borate buffer pH 11 and 6 ml. of toluene/isoamyl alcohol mixture (3:2 v/v) was added to each tube and (3 H-methyl) methoxytyramine was

extracted into the organic phase. After centrifugation 5 ml. of the organic phase was counted for tritium for 10 minutes in 10 ml. NE-260 micellar scintillator (Nuclear Enterprises, Edinburgh).

Standards and blanks

Coyle and Henry (1973) reported that over 80% of catecholamines were converted to their (^3H)-O-methylated derivatives by this method in the absence of tissue. The presence of tissue in the reaction mixture, however, reduced recovery (usually by less than 25% in brain tissue homogenized in 50 - 100 volumes).

Internal standards were, therefore, used containing 25 mg. noradrenaline (Sigma) or dopamine (Sigma) in 300 μl of the supernatant of a brain homogenate prepared from rat cortex and cerebellum. These brain regions were used because they contain relatively small concentrations of endogenous catecholamines.

Blank assays contained 300 μl of homogenizing acid, but no brain tissue.

Preparation of catechol-O-methyl transferase (Coyle and Henry, 1973)

100 g. of rat liver, homogenized in 4 volumes (w/v) of 0.9 M potassium chloride, was centrifuged at 78000 g. for 30 minutes. The supernatant was titrated to pH 5 with 1 M-acetic acid and the precipitate which formed after 20 minutes was removed by centrifugation. The supernatant was fractionated with ammonium sulphate.

a) 0 - 30% precipitate was discarded (16.4 g/100 ml. supernatant)

b) 30 - 55% precipitate (14.8 g/100 ml. supernatant)

was re-dissolved in 50 ml. of 1 mM sodium phosphate buffer pH 7. The enzyme solution was dialysed for 12 hours against 10^4 volumes of the same buffer containing 0.1 mM dithiothreitol and the final product centrifuged to remove precipitates, divided into 1 ml. aliquots and stored at -20°C . Under these conditions of storage activity is maintained for over 6 months.

Linearity of assay to added noradrenaline or dopamine

A homogenate was prepared from cortical and cerebellar tissue from a single rat. To five duplicate sets of tubes was added noradrenaline in amounts of 10, 20, 30, 50 and 100 mg. A further five sets of tubes received dopamine also in the amounts 10, 20, 30, 50, 100 mg. The assay was performed according to the method described and the results shown in Figures 8 and 9. (p 74, 75)

Turnover studies using α -methyl-p-tyrosine

Turnover measurement was based on the specific reversible inhibition of tyrosine hydroxylase in all brain regions by DL- α -methyl-p-tyrosine (methyl ester HCl) (Sigma M-8753) (Spector et al, 1965, Corrodi and Hanson, 1966), using the method of Brodie et al (1966).

The time course of dopamine and noradrenaline depletion in the amygdala after methyl-tyrosine injection was studied, using 20 unoperated rats weighing 180 - 200 g. 16 rats received a 2.5 ml. intraperitoneal injection of α -methyl-p-tyrosine dissolved in saline (250 mg/kg). A group of four rats received no injection and was used to provide basal levels of the monoamines. At 2, 3, 4 and 6 hours after injection groups of 4 rats were sacrificed and dissected in the usual manner. The right and left amygdala were dissected from each brain and tissue was pooled, weighed and stored in liquid nitrogen at -196°C for 1 - 4 days, until assayed for dopamine and noradrenaline concentration. The results are shown in Figure 10, from which it is apparent that the rate of loss of dopamine and noradrenaline in the rat amygdala is linear for up to 3 hours after the injection of methyl-tyrosine, but thereafter the rate slows down and there are large variations between animals. Therefore, in subsequent experiments to measure the effect of kindling on monoamine turnover in the amygdala and hippocampus, rats were killed $2\frac{1}{2}$ hours after injection of the inhibition.

A major influence on the rate of catecholamine turnover in the brain is stress and precautions were taken to minimize this during all stages of the procedure. For three days before the injection of methyl-tyrosine rats were handled for 5 minutes daily. On the morning of injection the animals were removed to a warm, quiet recording room with subdued lighting, at least one hour before injection. After injection animals were undisturbed until sacrifice and behaviourally they remained very quiet and inactive throughout the procedure.

Calculation of turnover rates of dopamine and noradrenaline

The method is based on the exponential decline of brain dopamine and noradrenaline levels following the inhibition of tyrosine hydroxylase by α -methyl-p-tyrosine. It is assumed that the conditions of a steady state exist and the rate of catecholamine synthesis is equal to the rate of efflux.

Thus the rate of synthesis (K) is given by the product of the rate constant of amine decline (k) and the basal catecholamine concentration $(C)_0$

$$K = k (C)_0 \quad (\text{Equation 1})$$

After blockade of tyrosine hydroxylase, the concentration of catecholamine obeys first order kinetics and declines at a rate proportional to concentration.

$$\frac{-d(C)}{dt} = k(C)$$

Integrating: $(C) = (C)_0 e^{-kt}$

which converts to: $\log(C) = \log(C)_0 - .434 kt$ (Equation 2)

where (C) = catecholamine level at time t

$(C)_0$ = catecholamine basal level.

A graph of $\log(C)$ against time gives a straight line with slope $0.434 k$. Substituting k in equation 1 gives K , the rate of efflux, which is assumed to be equal to the rate of synthesis of the amine.

Catecholamine concentrations have been measured at one fixed time ($2\frac{1}{2}$ hours) after α -methyl-tyrosine, after it had been shown that the fall in catecholamine concentration during that time was exponential (Figure 9).

Since $\log(C) = \log(C)_0 - .434 kt$ (Equation 2)

$$k = \frac{\log(C)_0 - \log(C)}{2.5 \times 0.434} \text{ hours}^{-1}$$

where $(C)_0$ = basal level of catecholamine

(C) = concentration $2\frac{1}{2}$ hours after α -methyl-tyrosine.

Turnover time is $1/k$ hours.

Turnover rate (rate of synthesis) = $k(C)_0 \mu\text{g/g/hour}$.

Comparison of turnover rates

The turnover rates found in any two groups of rats (say, kindled and sham-operated or kindled and unoperated controls, etc.) can be compared, using the student t statistic at $4n - 4$ degrees of freedom

Thus to compare group 1 and group 2:

$$t = \frac{\log C_1 - \log C_2 - \log c_1 + \log c_2}{\sqrt{e_1^2 + e_2^2 + e_3^2 + e_4^2}}$$

where C_1 = Basal amine concentration in group 1.

C_2 = Amine concentration $2\frac{1}{2}$ hours after α -methyl-tyrosine in group 1.

c_1 = Basal amine concentration in group 2.

c_2 = Amine concentration $2\frac{1}{2}$ hours after α -methyl-tyrosine in group 2.

e_1, e_2, e_3, e_4 are the standard errors of the mean levels $\log C_1, \log C_2, \log c_1, \log c_2$ respectively.

Reference: Saunders and Fleming (1957).

The estimation of cyclic GMP in slices from rat amygdala

Six kindled and six sham-operated rats were killed and the amygdalae dissected in the usual way. Tissue from two rats was pooled for cyclic GMP determinations providing six independent experiments: three with kindled and three with sham animals.

Preparation of slices

After dissection, the amygdala was immediately suspended in about 5 ml. ice-cold Krebs buffer pH 7.4 (previously oxygenated by gassing with 95% O_2 and 5% CO_2) with the following composition:

120 mM-NaCl 4.7 mM-KCl 25 mM- $NaHCO_3$ 1.2 mM- KH_2PO_4
1.0 mM- $CaCl_2$ 2.3 mM- $MgSO_4$ and 10 mM glucose.

Slices were prepared using a McIlwaine chopper, setting 350μ , applying two cuts in the vertical plane at right angles, and were then pre-incubated at 37°C for 45 minutes and, after two washes with fresh Krebs, each pool of slices was divided evenly into five separate flasks, each with a volume of about 2 mls. These were shaken gently for a further 15 minutes.

Incubation

1 ml. of Krebs was decanted from each flask and replaced by 1 ml. of the appropriate incubation medium. The final composition of the five incubation media was as follows:

1. Normal Krebs buffer: composition as above.
2. High Potassium Krebs: differed from normal Krebs in containing 5 mM-NaCl and 119.7 mM-KCl.
3. High Calcium/Krebs: differed from normal Krebs in containing 5 mM- CaCl_2 .
4. Haloperidol/Krebs: normal Krebs containing 10^{-5} M-Haloperidol ('Serenace' G.D. Searle Co.).
5. Dopamine/Krebs: normal Krebs containing 10^{-4} M-dopamine (3-Hydroxy- Tyramine HCl).

After 10 minutes incubation, the slices from each flask were divided into two separate tubes and the reaction was stopped by the rapid removal of the supernatant by suction and the addition of 1 ml. absolute ethanol to the remaining suspension of slices.

The suspension was allowed to evaporate to dryness slowly at 60°C and the tissue was homogenized in 100 μl of Tris EDTA buffer. After centrifugation for 15 minutes at 16000 g, the supernatant was withdrawn for the assay of cyclic GMP using a Radio Immunoassay Kit (Radiochemical Centre, Amersham, Bucks, England) and the protein concentration of the precipitate was measured by the method of Lowry *et al* (1951)

The Effect of Atropine on Kindling

The effect in two doses was tested on both the rate of kindling and the threshold of the after-discharge.

Experiment 1. Atropine Dose 25 mg/kg

17 rats were operated and from 6 - 14 days afterwards commenced kindling stimulation. One hour before each stimulation 9 rats received an intraperitoneal injection of atropine sulphate in a dose of 25 mg/kg body weight and 8 rats received an injection of 0.25 ml. saline. Atropine sulphate was dissolved in 0.9% (w/v) saline to give a solution containing 25 mg/ml.

Daily stimulation was applied in the standard way until the rats were fully kindled.

Experiment 2. Atropine Dose 2.5 mg/kg

These rats were treated in the same way as the previous groups, with the only exception that, due to departmental closure, a period of 25 - 33 days elapsed between the time of operating and the start of stimulation. Before each stimulation 7 rats received an intraperitoneal injection of 2.5 mg/kg atropine sulphate and 6 rats received 0.25 ml. saline.

The Effect of Atropine on After-discharge Threshold

7 rats which had been kindled in the previous experiments were used. 5 rats (numbers 1, 2, 3, 5, 8) had received atropine 25 mg/kg daily and 2 (numbers 10, 16) had received saline. Over four weeks elapsed between the completion of kindling and these further tests, for which the following procedure was adopted.

Always testing between 9 a.m. and noon, each rat was stimulated initially with a current of 40 μ a and then at 20 μ a increments, until an after-discharge was evoked. (20 μ a was considered to be the limit of sensitivity of the equipment available to us). On the following day stimulation was started at 40 μ a below the current intensity which had previously produced an after-discharge and the stimulus was continued in steps of 20 μ a until threshold was again reached.

Threshold determination was carried out on 7 successive days. On days 1, 2, 3, 5 and 7 rats received an intraperitoneal injection of 0.25 ml. 0.9% (w/v) saline: on day 4, 25 mg/kg atropine and on day 6, 2.5 mg/kg

atropine in saline. All injections were given 1 hour before threshold determination.

Muscarinic Receptor Numbers

Muscarinic receptor numbers were assayed in different brain regions of kindled, sham-operated and unoperated rats, using the specific agonist quinuclidinyl benzilate (QNB) in a radioligand assay essentially as described by Hulme et al (1976) and modified slightly by Davies and Verth (1978).

The muscarinic receptor is assayed by measuring the binding of radio-labelled QNB to the particulate matter of a brain homogenate. Specific binding is measured as the difference in the amount of binding of labelled QNB in the presence and absence of a large excess of unlabelled antagonist, atropine. Free and bound drug are separated by centrifugation.

Animals

In the first experiment using 5 kindled and 5 matched sham-operated rats, the amygdala, hypothalamus, hippocampus, reticular formation and frontoparietal cortex were dissected under a dissecting microscope, and with reference to the illustrations of the Rat Atlas. In a second group of 5 kindled and 7 sham-operated rats and 5 unoperated controls, only the right and left amygdala were dissected.

Kindled rats had received their last convulsion 4 weeks before sacrifice.

Animals were killed, their brains removed and dissected as previously described. Tissue was stored in liquid nitrogen for up to 3 months, until required for assay. It had previously been shown that storage of human and rat brain in this manner did not cause a reduction in the number of muscarinic sites.

Brain tissue was homogenized in Krebs-Hensleith buffer at a concentration of 5 mg. tissue per ml. of buffer. An aliquot of the homogenate was set aside for protein determination by the method of Lowry et al (1951). It was shown that homogenate of brain prepared at 4°C began to lose QNB binding sites after about 2 hours, even when left on ice. All assays were, therefore, done within this time.

Taking great care by frequent whirling mixing that settling out did not occur in the homogenate during pipetting, 1 ml. aliquots were transferred to Eppendorf tubes. Each brain region was assayed in triplicate in the

presence and absence of 10 μ M atropine sulphate, so 6 aliquots were required from each homogenate.

To three of the tubes was added 10 μ l of ^3H -QNB (16.4 Ci/mMol) to give a final concentration of 10 nM, the binding in these samples representing total binding. To the second triad of tubes was added 10 μ l of atropine sulphate (final concentration 10 μ M) in addition to ^3H -QNB (10 nM). These tubes measured non-specific binding. The tubes were incubated at 30°C for 20 minutes and the reaction stopped by centrifugation at 12000 g. for 3 minutes (Koolspin refrigerated centrifuge). The pellet was rapidly and superficially washed three times in a large excess of ice-cold Krebs-Hensleit buffer and then dissolved in 300 μ l Protosol (New England Nuclear) before transfer to a scintillation vial in 2 ml. ethanol. 10 ml. toluene scintillant was added to the tube, shaken vigorously and allowed to stand for two hours, to let chemiluminescence DECAY. The radioactivity was counted for tritium at a counting efficiency of 36%.

The number of receptors is expressed in terms of p moles QNB bound per mg. protein. Since one QNB molecule is assumed to bind to each receptor, this gives p moles receptor per mg. protein.

Saturability of Specific QNB Binding to Rat Brain Preparations

To confirm that 10 nM QNB was a saturating concentration of the ligand the specific binding of QNB at a range of concentrations was measured in a homogenate of rat parietal cortex, by the method described above.

The following concentrations of QNB were employed: 50 nM, 25 nM, 10 nM, 7 nM, 5 nM, 3.3 nM, 2.5 nM, 1 nM, 0.5 nM and the results are shown in Figure 11.

Solutions Used for QNB Binding Assays

Krebs-Hensleit Buffer

NaCl	0.9% (w/v) (.154 M)	KCl	1.15 % (w/v) (.154 M)
CaCl ₂	1.22% (w/v) (.11 M)	KH ₂ PO ₄	2.11% (w/v) (.154 M)
MgSO ₄	7H ₂ O 3.8% (w/v) (.154 M)	NaHCO ₃	1.3% (w/v) (.15 M)

(Sodium bicarbonate solution was gassed with 5% CO₂/air mixture for 1 hour before mixing with the other solutions).

Toluene Scintillant

4. 25 g. PPO and 0.11 g. POPOP dissolved in 1 l. Toluene.

The Measurement of Sodium Dependent High Affinity Choline Uptake in Regions of the Rat Brain

High affinity choline uptake was measured by a modification (L. Smart unpublished) of the methods described by Yamamura and Snyder (1973) and Simon et al (1976).

Animals

6 kindled rats, 6 sham-operated rats and 8 unoperated controls were used. The rats were of the same age and allocation to their respective groups was prospective. Of the kindled group, 6 rats became fully kindled and the rest were discarded. The sham-operated rats received no stimulations and they and the unoperated controls were handled regularly.

About four weeks after the last convulsion of the kindled rats, animals were sacrificed and choline uptake measured on the same day, so that tissue was not stored in the frozen state.

Fronto-parietal cortex, hippocampus striatum and amygdala were bilaterally dissected in the usual way, and placed in weighed ependorfs, kept cool on ice and re-weighed.

Tissue was homogenized in a tube using a ground glass tipped homogenizer for 45 seconds at 1000 - 2000 rpm in 0.32 M sucrose in the ratio of 10 mg. wet weight: 1 ml sucrose. Tubes were surrounded by ice at all stages. The homogenate was centrifuged at 1000 g. for 5 minutes at 4°C in C 14 glass tubes, to yield a debris-free P 1 fraction containing synaptosomes. An aliquot of the supernatant was removed for protein assay by the method of Lowry^{et al (1951)}. Choline uptake was measured in triplicate samples in the presence of both a sodium-containing and a sodium-free buffer (6 tubes for each homogenate).

500 µl of buffer and 100 µl of brain homogenate were preincubated at 30°C for 5 minutes in ependorf tubes. The reaction was initiated by the addition of

20 μ l ^3H -choline to a final concentration of 0.6 μM and after 4 minutes terminated by the addition of 300 μ l ice-cold hemicholinium to a final concentration of 10^{-3} M. Tubes were mixed immediately, left on ice for 4 minutes and centrifuged at 16000 g. on a Koolspin centrifuge at 0°C for 4 minutes. The supernatant was discarded and the pellet washed rapidly and superficially three times with a large volume of ice-cold 0.9% (w/v) saline. The protein was dissolved in 300 μ l. Protosol and the entire Eppendorf tube and its contents tipped into a scintillation vial, to which was added 10 ml. of toluene scintillation liquid.

The activity of tritium was estimated by scintillation counting at an overall efficiency of 34.8%.

Solutions Used for Choline Uptake

Krebs-Ringer phosphate buffer:

NaCl	126 mM	KCl	4.75 mM	
CaCl	1.27 mM	Na ₂ HPO ₄ 2H ₂ O	15.8 mM	(ADJUSTED to pH 7.5 with HCl)
MgCl ₂	1.42 mM			

Sodium Free Buffer

Sucrose	252 mM
KCl	4.75 mM
CaCl ₂	1.27 mM
MgCl ₂	1.42 mM
Tris-PO ₄	15.8 mM pH 7.4 at 30°C .

Scintillant

Toluene 1 l. + PPO 4.25 g.

The Saturability of Choline Uptake

Sodium dependent high affinity choline uptake was measured in a crude debris-free homogenate (P₁ fraction) of rat cortex, using the following range of final choline concentrations:

0.04 0.1 0.2 0.4 0.6 0.8 μM

The reaction mixture contained 0.12 mg. protein. The choline uptake is

shown in Figure 12.

The Effect of Protein Concentration in the Assay of Choline Uptake

A single rat cortex was dissected and homogenized to give a homogenate containing 20 mg. in 1 ml.

This was further diluted to give a series of concentrations which, after estimation of protein by the Lowry method, were found to contain; 92, 276, 368, 460, 552 μ g protein.

Sodium-dependent high affinity choline uptake using differing concentrations of protein in the assay medium is shown in Figure 14.

The Effect of Pentobarbitone Treatment of Rats on Choline Uptake Assayed in the Hippocampus: Test for Post-mortem Loss of Activity

To confirm that the rate of choline uptake which was being measured was reflecting cholinergic activity in vivo, the effect of pre-treating rats with pentobarbitone on choline uptake was studied. Klem and Kuhar (1979) showed that activity related changes in choline uptake are extremely labile and undergo a reversal in post-mortem brain tissue after about 10 minutes at 25°C. At 0 - 4°C the activity related changes are more enduring. To verify that the assay conditions were adequate for the detection of small changes in cholinergic activity, 5 rats were injected intraperitoneally with pentobarbitone sodium (Sagatal - May and Baker) (65 mg/kg) and 4 rats with 0.9% (w/v) saline 30 minutes before decapitation. The high affinity choline uptake was measured in the combined right and left hippocampus from each of the rats and results are given in Table 20.

Estimation of Acetylcholine Esterase Activity

Acetylcholine esterase was assayed using a modification of the method of Fonnum (1969).

Animals

A total of 15 rats was used. Four animals were unoperated controls. Of the 11 operated animals five had been fully kindled after an average of 14 (Range 11 - 18) daily stimulations. Six rats formed the operated control group and these had received no more than one or two stimulations, which were discontinued on account of faulty electrodes or high threshold. A period of two weeks elapsed between the time of the last convulsion in the kindled group and the time of assay.

Preparation of Tissue Homogenate

Rats were kindled and the brains removed and dissected as previously described. The amygdala, striatum and parietal cortex were removed bilaterally and weighed. Samples were homogenized in 1 mM EDTA buffer pH 7 containing Triton X100 using 1 ml. buffer for 5 mg. tissue. The homogenization technique was as described for the ATPase assay and the crude homogenate was used in the assay.

Acetylcholine Esterase Assay

5 μ l of an incubation medium and 5 μ l of tissue homogenate were mixed in a C 14 glass tube and incubated for 20 minutes at 37°C, with agitation in water bath. The incubation medium, which was mixed immediately before use, contained:

0.5 mM (1 - C¹⁴) -acetylcholine
20 mM-NaH₂ PO₄ buffer pH 7.2
Albumin 1.6 mg/ml.

The reaction was stopped by adding 2 ml. of ice-cold 10 mM-NaH₂ PO₄ buffer pH 7.4. The residue of radioactive unhydrolysed acetylcholine was separated from the released labelled acetate by fractionation against kalignost-toluene, which takes up acetylcholine but not free acetate. 1 ml. kalignost-toluene was added, mixed and centrifuged (MSE mistral 2 L) for 30 minutes at 28000 rpm). The upper kalignost layer was suctioned off and discarded. 1 ml. of fresh kalignost was added for a second extraction and the upper layer again discarded.

A 1 ml. aliquot of the lower layer was transferred to a scintillation vial and 10 ml. Aquasol scintillant was added. ¹⁴C activity was counted for 10 minutes at an efficiency of 84%. (Nuclear Chicago Mark II liquid scintillation system). Acetylcholine esterase activity was expressed as μ moles acetate liberated/hour/g wet weight.

Solutions

Homogenization buffer: 1 mM-EDTA in H₂O, pH
adjusted to 7.0 with NaOH.

Kalignost/Toluene

3.75 g. of sodium tetra-phenylboron dissolved in 250 ml. toluene.

Determination of ATPase Activity

Total ATPase activity was measured by the method of Skou (1957) as modified by Norby (1971). Na/K ATPase concerned with the ionic pump mechanism of the cell was estimated by performing the assay in the presence and absence of ouabain. The addition of ouabain to a final concentration of 10^{-3} M in the incubation mixture completely and specifically inhibits Na/K (pump) ATPase (Skou et al, 1969), Bonting et al, 1963).

Animals

Twelve rats were used. Six animals had been fully kindled an average of 2.8 months before sacrifice (Range 1.5 to 6 months). The control group was six rats which had been operated at approximately the same time as the kindled group. Two control animals had received no stimuli and four animals had either shown no after-discharge with the first few stimulations or had failed to kindle beyond Stage 1 - 2 despite repeated stimulation.

Preparation of Tissue Homogenate

Rats were killed and the brain areas parietal cortex, amygdala and corpus striatum were dissected and weighed, as previously described. 20 mM-Tris buffer pH 7.4 (1 ml. buffer: 10 mg. brain tissue) was added and the tissue was homogenized with a loose fitting glass homogenizer with a variable clearance of 0.1 - 0.125 mm. for two periods of 30 seconds, separated by a 30-second pause on ice, at a speed ranging from 1000 - 2000 rpm using a Tri-R Stir-R model K41. The homogenization tube was held jacketed with ice throughout. The crude homogenate was used without further purification.

To the Ependorf tubes was added in the following order: 700 μ l Skou medium (to give final concentrations of Na 130 mM, K 20 mM, Mg 3 mM, . ATP 3 mM), 100 μ l of water, to give final concentration of 10^{-4} M, 100 μ l homogenate.

All assays were done in triplicate and each homogenate was assayed in the presence and absence of ouabain (i.e. 6 tubes from each homogenate).

The Ependorf tubes were pre-incubated in a water bath at 37°C for 10 minutes before the addition of 100 μ l ATP (final concentration 3 mM) pre-warmed to 37°C.

The reaction was allowed to run for 20 minutes at 37°C and was halted by the addition of 200 μ l ice-cold 4 molar perchloric acid.

The Ependorfs were left on ice for 20 minutes to allow protein to precipitate before centrifugation at 15000 g. for 4 minutes (Koolspin (Burkard) refrigerated centrifuge).

Measurement of Inorganic Phosphate (Method of Atkinson et al, 1973)

500 μ l samples of supernatant were transferred to a C 14 glass tube. For phosphate assay duplicate samples were taken from each supernatant and 1 ml. cirrasol molybdate mixture was added. After 10 minutes the optical density of the resulting yellow colour was read at 390 nm on a Gilford 250 Spectrophotometer.

Standards and controls

1. Phosphate standards:

Using KH_2PO_4 as phosphate source standards were prepared using 0, .2 mM, .5 mM, 1 mM, 1.5 mM, 2 mM.

2. 5 Protein blanks.

These tubes did not receive ATP.

3. 5 tubes to assess ATP hydrolysis.

These tubes did not receive the homogenate containing ATPase.

Protein Estimation

By the method of Lowry et al, 1951.

Because Tris buffer was found to interfere slightly with the Lowry method, protein standards were prepared in Tris and not water for this assay.

Expression of Results

ATPase was expressed as μ moles $\text{Pi}/\text{mg. protein/hour}$.

Activity measured in the absence of ouabain was termed total ATPase and activity insensitive to ouabain termed Mg^{++} ATPase. The difference was Na/K ATPase.

Solutions used in ATPase Assay

Skou Medium: 130 mM NaCl, 20 mM KCl
3 mM MgCl_2 6H₂O, 20 mM Tris buffer
pH 7.4 at 37°C.

Tris buffer replaced 30 mM Histidine since the latter interfered with the Lowry estimation.

Tris Buffer: Trizma - HCl 2.29 g/l
 Trizma - base .664 g/l.

Cirrasol - Acid Molybdate Solution:

Solution A: Cirrasol ALN-WF 5% was prepared monthly by warming in distilled water at 37°C.

Solution B: Acid Molybdate was $(\text{NH}_4)_2 \text{MoO}_7 \cdot 4\text{H}_2\text{O}$. A 2% solution in 1.8 M H_2SO_4 was stored in dark polythene bottles at 4°C.

The Cirrasol - Acid Molybdate Reagent was prepared by mixing Solution A 10 Vol., Solution B 25 Vol., water 65 Vol.

Protein Estimation by the Method of Lowry et al (1951)

<u>Reagents</u>	A	2% Na_2CO_3 in 0.1 NaOH
	B	i) 1% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ii) 2% NaK Tartrate
	C	50 parts A + 1 part B (equal vols, (i) + (ii))
	D	Folin Ciocalteu reagent diluted 1 + 1.5.

Preparation of Standards

0.125 g. of serum albumin (Bovine albumin fraction 5 powder: Sigma) is freshly dissolved in 50 ml. distilled water. Fractions of 0.1 to 0.5 ml. are diluted to 5 ml. with water so that 0.3 ml. aliquots of these dilutions give a series of standards containing 15, 30, 45, 60 and 75 μg protein in 0.3 ml.

Protein Estimation

3 ml. of solution C is added to 0.3 ml. of the standards, blanks and test solutions and the mixture allowed to stand for 15 minutes after shaking. 0.3 ml. solution D is then added to each tube and mixed immediately. After 30 minutes the blue colouration is measured by reading the optical density at 750 nanometers using visible light source and red filter on a Gilford Spectrophotometer 250.

2 - 3 RESULTS

(a) Noradrenaline and Dopamine Concentration and Turnover after Kindling

(i) Linearity of the assay to added noradrenaline and dopamine

Figures 8 and 9 demonstrate the linearity of the assay for both noradrenaline and dopamine up to concentrations of over 50 ng which is well in excess of the amount of catecholamine present in any of the assays in kindled rat brains.

(ii) Turnover studies using methyltyrosine

In Figure 10 the rate of loss of noradrenaline and dopamine from the amygdala following a single I. P. injection of methyltyrosine is shown.

The rate is linear for 2½ hours for both catecholamines, indicating first order kinetics. During this period the inhibition of tyrosine hydroxylase is complete and since no synthesis of transmitter can occur the rate of loss is equal to rate of utilization - the turnover rate.

In subsequent experiments using kindled amygdalae animals were all killed 2½ hours after methyltyrosine injection.

Using the data from Figure 10 a 2 hour rate constant has been calculated for both noradrenaline and dopamine. These are given in Table 4 and Table 5.

The turnover rate (rate of synthesis) of noradrenaline is 0.19 µg/g/hour and of dopamine 0.23 µg/g/hour.

Noradrenaline concentration in 6 brain regions of kindled, sham-operated and unoperated animals

Table 6 shows the concentration of noradrenaline in the right and left amygdala, hippocampus and striatum of kindled, sham-operated and unoperated animals.

The noradrenaline concentration was significantly lower in the left amygdala of kindled animals compared to the right amygdala ($P < .05$). The significance of this observation is considerably diminished by the absence of any other significant contrasts. Thus the left kindled amygdala does not differ from left sham or unoperated amygdala.

FIGURE 8
LINEARITY OF COMT REACTION WITH VARIOUS
CONCENTRATIONS OF NORADRENALINE

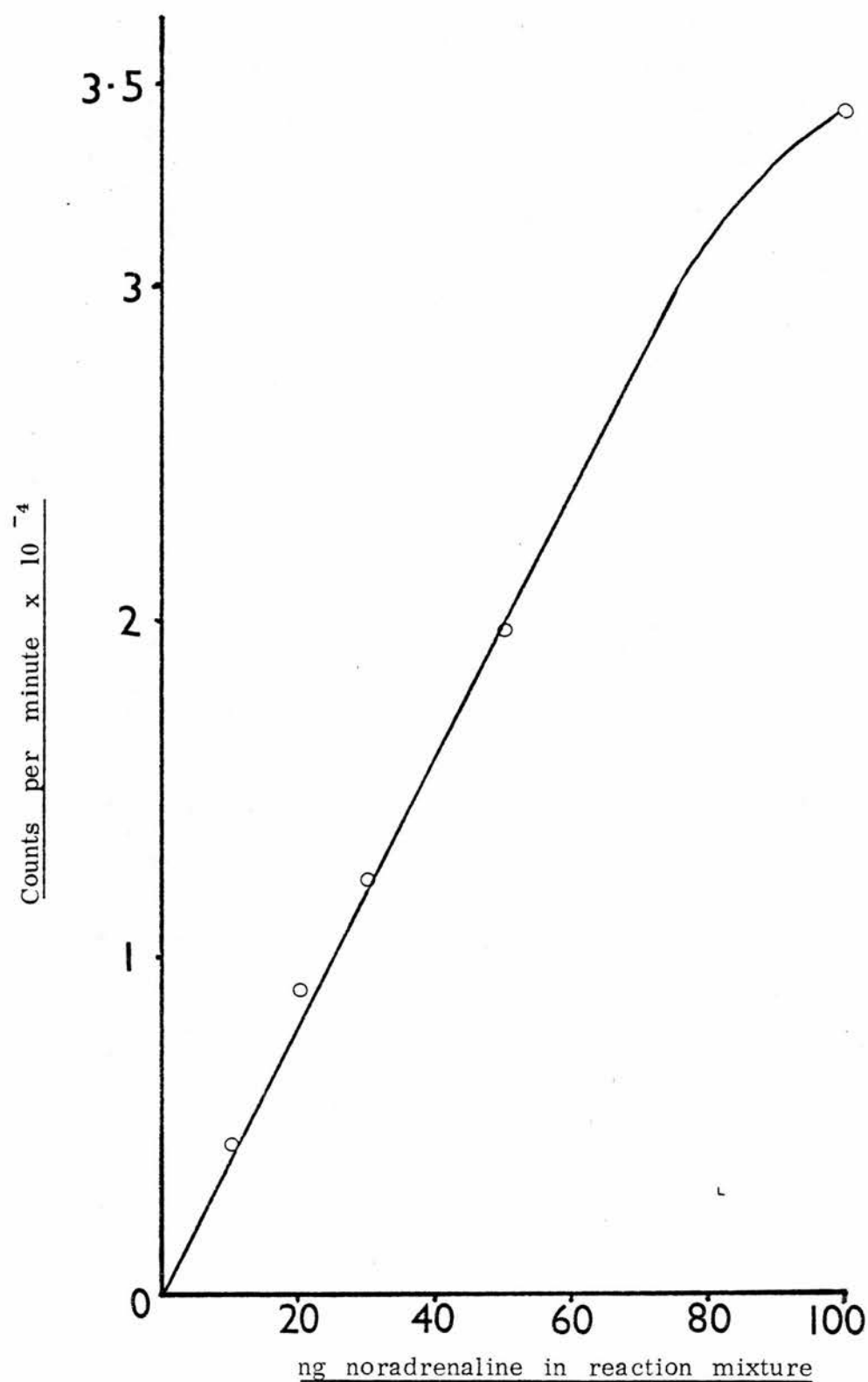


FIGURE 9

LINEARITY OF COMT REACTION
WITH VARIOUS CONCENTRATIONS
OF DOPAMINE

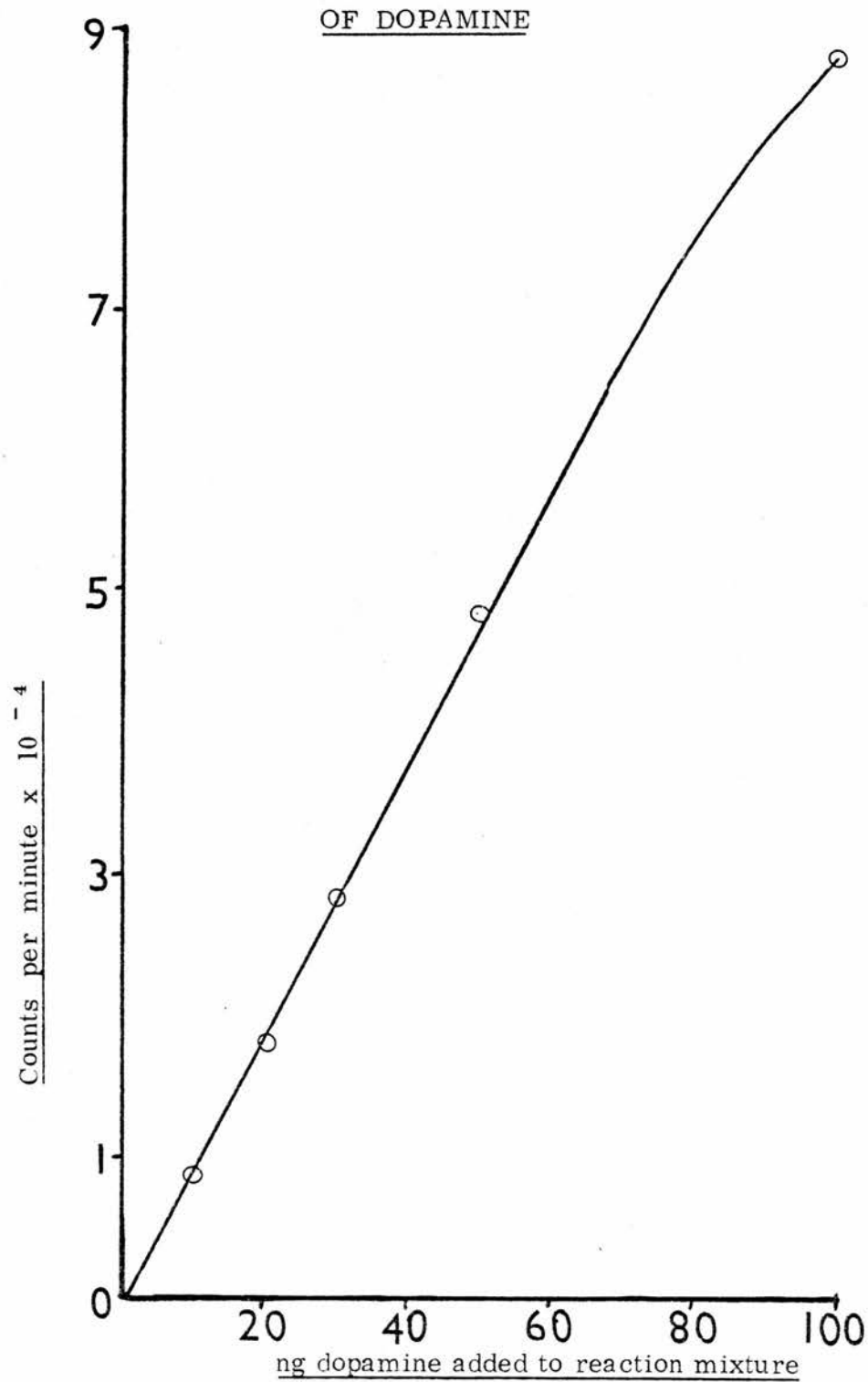


FIGURE 10 THE CONCENTRATION OF NORADRENALINE AND DOPAMINE IN RAT AMYGDALA

AT VARIOUS TIMES AFTER THE INJECTION OF α -METHYLTYROSINE
(2.50 mg/Kg) I. P.

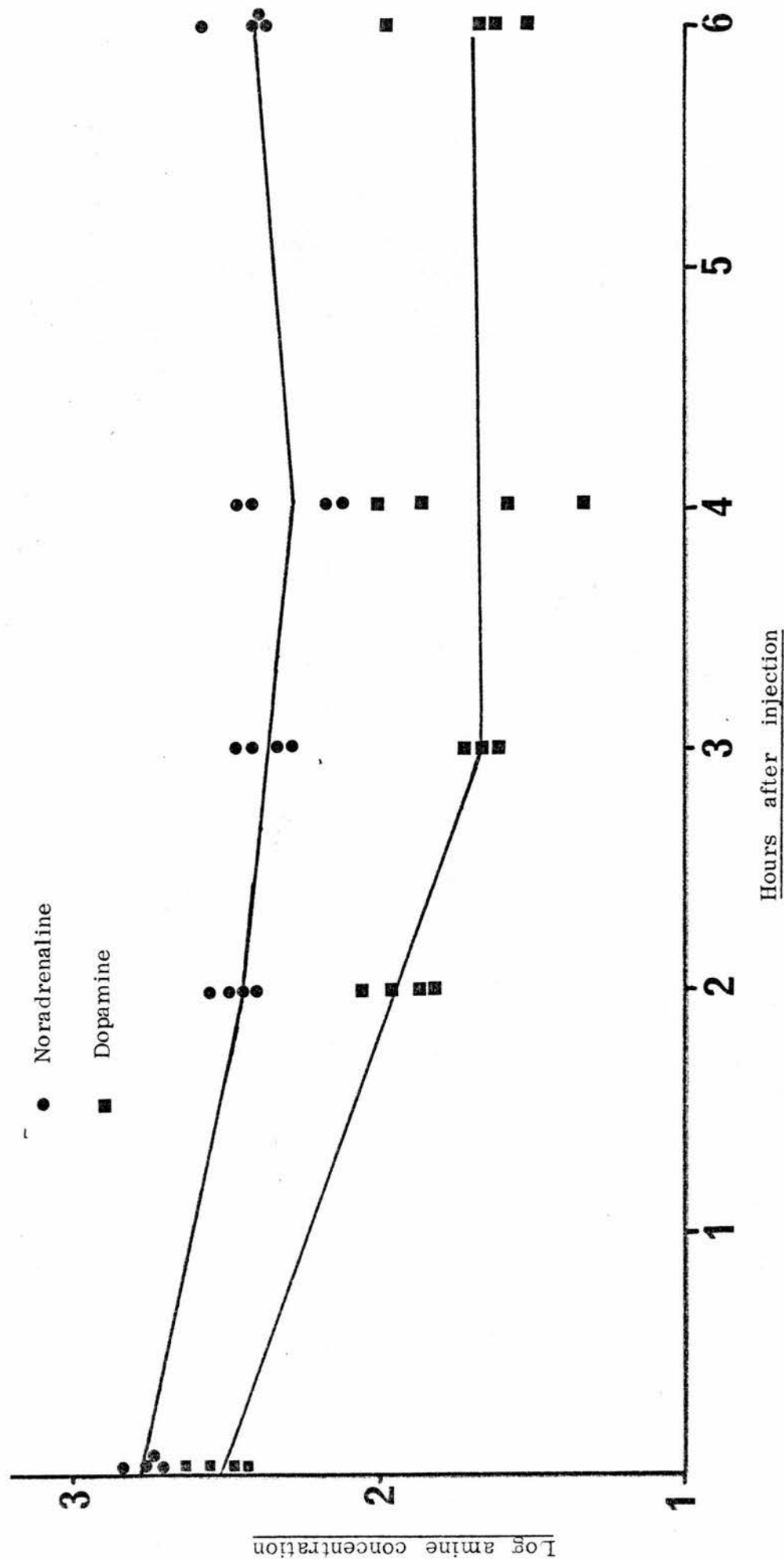


TABLE 4

RATE OF SYNTHESIS OF NORADRENALINE (NE)
IN RAT AMYGDALA
(From data used to construct figure 10)

Time after α Methyltyrosine Injection	Concentration of Noradrenaline ng/g wet wt. Mean \pm Sem	Log [NE]	Standard Error of Mean Log [NE]	(SEM) ²
0 Hours n = 5	581 \pm 35.3	2.76	.0276	7.1 \times 10 ⁻⁴
2 Hours n = 4	303 \pm 23.2	2.48	.0333	11.1 \times 10 ⁻⁴
3 Hours n = 4	246 \pm 23.6			
4 Hours n = 4	210 \pm 41.5			
6 Hours n = 4	273 \pm 40.1			

$$2 \text{ Hour rate constant } k = \frac{2.76 - 2.48}{2 \times .434} = \frac{0.33}{\pm .043}$$

$$\text{Turnover Time} = 1/k = 3.1 \text{ hours}$$

$$\text{Turnover Rate} = k [\text{NE}]_0 = 0.19 \text{ } \mu\text{g/g/hour}$$

(Rate of Synthesis)

TABLE 5

RATE OF SYNTHESIS OF DOPAMINE
IN RAT AMYGDALA
(From data used to construct figure 10)

Time after α Methyl- tyrosine injection	Concentration of Dopamine ng/g wet weight mean \pm Sem	Log[DA]	Standard Error of Mean Log [DA]	(SEM) ²
0 Hours n = 4	344 \pm 39.7	2.53	.0466	21.7 \times 10 ⁻⁴
2 Hours n = 4	90.0 \pm 11.7	1.94	.0535	28.6 \times 10 ⁻⁴
3 Hours n = 4	47.3 \pm 2.3			
4 Hours n = 4	60.3 \pm 18.2			
6 Hours n = 4	55.7 \pm 14.7			

$$2 \text{ Hour rate constant of amine efflux } k = \frac{2.53 - 1.94}{2 \times .434}$$

$$= .68 \pm .071 \text{ (SEM)}$$

$$\text{Turnover time } 1/k = 1.5 \text{ hours}$$

$$\text{Rate of Dopamine Synthesis} = k [\text{DA}]_0$$

$$= 0.234 \text{ } \mu\text{g/hour/g wet weight.}$$

TABLE 6

NORADRENALINE CONCENTRATIONS IN
VARIOUS BRAIN REGIONS OF KINDLED
SHAM OPERATED AND UNOPERATED RATS
 (ng/g wet weight)

	Kindled			Sham Operated			Unoperated		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
LEFT AMYGDALA	543	27.5	6	553	24.3	6	540	68.4	6
RIGHT AMYGDALA	632	78.5	5	616	113.3	4	585	96.2	6
L. HIPPOCAMPUS	819	192.	9	755	191.	9	731	136.	9
R. HIPPOCAMPUS	836	238.	9	848	161.	9	778	123.	9
LEFT STRIATUM	396	114.	7	410	62.	7	Not done		
RIGHT STRIATUM	417	136.6	6	434	71.1	6	Not done		

The differences in Noradrenaline concentration between right and left Amygdala in kindled rats is significant ($p < .05$ two tailed student t test) other contrasts within each brain region do not reach significance.

88.

Dopamine concentration in six brain regions of kindled, sham-operated and unoperated animals

Table 7 shows the concentration of dopamine in the right and left amygdala, hippocampus and striatum of kindled, sham-operated and unoperated animals. Neither kindling nor the operation had any effect on dopamine basal levels.

The rate of synthesis of noradrenaline in kindled, sham-operated and unoperated rats

The rate of synthesis of noradrenaline is given in Table 8 for the amygdala and Table 9 for the hippocampus. In neither region does the rate of noradrenaline alter as a result of kindling.

The rate of synthesis of dopamine in kindled, sham-operated and unoperated rats

The rate of synthesis of dopamine is given in Table 10 for the amygdala and Table 11 for the hippocampus.

In the amygdala the turnover rate of dopamine is increased in the left (stimulated) amygdala compared to the right side ($P < .02$). No difference between right and left sides or between groups in the hippocampus.

TABLE 7

DOPAMINE CONCENTRATIONS IN VARIOUS
BRAIN REGIONS OF KINDLED,
SHAM-OPERATED AND UNOPERATED RATS
 (ng/g wet weight)

	Kindled			Sham Operated			Unoperated		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
LEFT AMYGDALA	328	89.5	6	399	104.8	6	294	44.7	6
RIGHT AMYGDALA	331	52.5	6	353	103.8	6	322	56.	6
LEFT HIPPOCAMPUS	46	17.3	9	45.4	16.1	8	50.4	19.0	7
RIGHT HIPPOCAMPUS	50.5	23.3	8	59.5	20.0	8	52.3	17.4	9
LEFT STRIATUM	16731 \pm 4592 7			16127 \pm 3828 7			Not done		
RIGHT STRIATUM	18406 \pm 4148 6			18475 \pm 4035 6			Not done		

The differences between right and left sides and between kindled, sham operated and unoperated controls are not significant at the 5% level in any of the brain regions.

RATE OF NORADRENALINE SYNTHESIS IN THE AMYGDALA OF
KINDLED, SHAM-OPERATED AND UNOPERATED RATS

	<u>Time after a-Methyltyrosine Injection</u>	<u>Noradrenaline Concentration</u> ng/g					
		<u>Left Amygdala</u>			<u>Right Amygdala</u>		
		Mean	SD	N	Mean	SD	N
Kindled	Basal	543	27.5	6	632	78.5	5
	2½ hours	345	65.6	5	282	141.6	5
Sham	Basal	553	24.3	6	616	113.3	4
	2½ hours	235	102	4	218	51.3	5
Unoperated	Basal	540	68.4	6	588	85.2	6
	2½ hours	374	75.7	5	397	130	5

	<u>Rate Constant</u> (Hours) ⁻¹			<u>Turnover Time</u> (Hours)	<u>Turnover Rate</u> k [C] ₀ μg/g/Hour
	k	±	SEM	1/k	
R. Kindled	0.47	±	0.024	2.2	0.30
R. Sham-operated	0.57	±	0.07	1.8	0.35
R. Unoperated	0.24	±	0.10	4.1	0.14
L. Kindled	0.23	±	0.04	4.3	0.13
L. Sham-operated	0.46	±	0.14	2.2	0.26
L. Unoperated	0.20	±	0.10	5.1	0.11

Differences between right and left and between kindled, sham and unoperated controls are not significant at 5% level (Student t).

RATE OF NORADRENALINE SYNTHESIS IN THE HIPPOCAMPUS OF
KINDLED, SHAM-OPERATED AND UNOPERATED RATS

	<u>Time after</u> <u>a-Methyltyrosine</u> <u>Injection</u>	<u>Noradrenaline Concentration</u> <u>ng/g</u>					
		<u>Left Hippocampus</u>			<u>Right Hippocampus</u>		
		Mean	SD	N	Mean	SD	N
Kindled	Basal	819	192	9	836	238	9
	2½ hours	300	68.5	6	368	81.2	6
Sham	Basal	755	191	9	848	161	9
	2½ hours	251	58.9	6	261	44.4	6
Unoperated	Basal	731	136	9	778	123	9
	2½ hours	277	69.4	6	294	62	6

	<u>Rate Constant</u> <u>(Hours)⁻¹</u>			<u>Turnover</u> <u>Time</u> <u>(Hours)</u>	<u>Turnover</u> <u>Rate</u>
	k	±	SEM	1/k	k [C] ₀
					μg/g/Hour
R. Kindled	0.32	±	.062	3.1	0.27
R. Sham-operated	0.47	±	.044	2.1	0.40
R. Unoperated	0.40	±	.049	2.5	0.31
L. Kindled	0.40	±	.057	2.5	0.33
L. Sham-operated	0.43	±	.062	2.3	0.33
L. Unoperated	0.39	±	.058	2.6	0.29

Differences in turnover rate between right and left sides in kindled, sham-operated and unoperated groups are not significant ($P > 0.5$).

RATE OF DOPAMINE SYNTHESIS IN THE AMYGDALA OF KINDLED,
SHAM-OPERATED AND UNOPERATED RATS

	<u>Time after a-Methyltyrosine Injection</u>	<u>Dopamine Concentration</u> ng/g					
		<u>Left Amygdala</u>			<u>Right Amygdala</u>		
		Mean	SD	N	Mean	SD	N
Kindled	Basal	328	89.5	6	331	52.5	6
	2½ hours	83	12.6	6	151	34.4	6
Sham	Basal	399	105	6	353	104	6
	2½ hours	92	80	6	145	77	6
Unoperated	Basal	294	44.7	6	322	56	6
	2½ hours	107	22.1	6	111	74.5	6

	<u>Rate Constant</u> (Hours) ⁻¹			<u>Turnover</u> <u>Time</u> (Hours)	<u>Turnover</u> <u>Rate</u> k [C] ₀ μg/g/Hour
	k	±	SEM	1/k	
R. Kindled	0.33	±	0.063	3.0	0.11
R. Sham-operated	0.40	±	0.086	2.5	0.14
R. Unoperated	0.49	±	0.103	2.1	0.16
I. Kindled	0.56	±	0.061	1.8	0.18
I. Sham-operated	0.70	±	0.099	1.5	0.28
I. Unoperated	0.41	±	0.51	2.4	0.12

The difference between rate of synthesis in r. kindled and l. kindled is significant ($P < .02$ two tailed student t). Other contrasts between right and left are not significant and comparisons between groups are not significant.

RATE OF DOPAMINE SYNTHESIS IN THE HIPPOCAMPUS OF KINDLED,
SHAM-OPERATED AND UNOPERATED RATS

	<u>Time after α-Methyltyrosine Injection</u>	<u>Dopamine Concentration</u> <u>ng/g</u>					
		<u>Left Hippocampus</u>			<u>Right Hippocampus</u>		
		Mean	SD	N	Mean	SD	N
Kindled	Basal	46.0	17.3	9	50.5	23.3	8
	2½ hours	22.0	5.4	6	24.5	3.5	6
Sham	Basal	45.4	16.1	8	59.5	20.0	8
	2½ hours	19	3.1	6	24.2	4.8	6
Unoperated	Basal	50.4	19.0	7	52.3	17.4	9
	2½ hours	22.2	5.1	6	28.2	4.8	6

	<u>Rate Constant</u> <u>(Hours)⁻¹</u>			<u>Turnover</u> <u>Time</u> <u>(Hours)</u>	<u>Turnover</u> <u>Rate</u> <u>k [C]₀</u> <u>μg/g/Hour</u>
	k	±	SEM	1/k	
R. Kindled	.19	±	.083	5.2	.010
R. Sham-operated	.36	±	.063	2.8	.021
R. Unoperated	.30	±	.058	3.4	.016
L. Kindled	.29	±	.067	3.5	.013
L. Sham-operated	.32	±	.061	3.1	.015
L. Unoperated	.32	±	.082	3.1	.016

Differences between right and left and between kindled, sham and unoperated are not significant ($P > .05$ student t).

c GMP RESULTS

From the kindling group six rats had become fully kindled. These required an average of 13.7 stimulations (Range 7 - 18). Behavioural and electrographic changes were similar to those described elsewhere. No spontaneous convulsions were seen among the kindled group of rats during the time between the completion of kindling and the experiments. Kindled rats were killed at least 12 weeks after the time of their last convulsion.

The results of cyclic GMP estimations in the right and left amygdala of the 6 kindled and 6 sham-operated rats are shown in Table 12.

In the analysis of variance of the data in Table 12, the four main factors considered were:

- G. Group (sham and kindled)
- T. Treatment (Basal, K^+ , Ca^{++} , Dopamine, Haloperidol)
- S. Sides (right and left)
- E. Experiment (I, II, III).

The interaction of all four factors and the second order interactions did not reach the 5% level of significance. Of the first order interactions only $G \times S$ was significant ($F = 30.55$ $P < .001$). I proceeded to a breakdown analysis by Group (sham and kindled) and the results are displayed in Table 13 and Table 14.

In the data from sham animals (Table 14) all secondary and primary interactions were not significant and of the main factors only the effect of treatment was significant ($F = 7.296$ $P < .01$). Inspection of the results in Table 12 shows a rise in cyclic GMP above basal level in all groups in every experiment. In the sham groups there was no difference between right and left sides and the results did not differ significantly between experiments.

Analysis of the data in Table 12 from kindled rats is given in Table 13. The four treatments tested caused no significant change in cyclic GMP levels. There was a significant difference between right and left amygdala ($F = 52.29$ $P < .001$) and no significant difference between experiments.

In both the left and the right amygdala the basal levels of cyclic GMP tended to be higher in the kindled than in sham animals (Table 12) and this increase was significant (Wilcoxon Rank Sum Test $P < .05$).

TABLE 12

CYCLIC GMP CONCENTRATION IN AMYGDALA (pm cGMP/mg PROTEIN)

(Each value is the mean for two animals pooled in the same experiment)

	<u>Experiment</u>	<u>Basal</u>	<u>K⁺</u>	<u>Ca⁺⁺</u>	<u>Dopamine</u>	<u>Haloperidol</u>
<u>SHAM</u>	<u>Left</u>					
	I	.77	1.44	1.71	2.14	1.66
	II	1.01	1.77	2.22	3.83	2.39
	III	1.29	1.80	1.72	2.56	2.89
	<u>Right</u>					
	I	1.33	2.48	1.52	2.26	2.37
<u>KINDLED</u>	II	1.20	1.28	1.62	1.66	2.04
	III	.78	1.42	1.02	3.06	1.37
	<u>Left</u>					
	I	1.03	1.22	2.26	1.93	.79
	II	1.70	1.66	1.30	1.22	2.28
	III	1.43	1.83	1.16	1.32	1.67
<u>Right</u>	I	1.28	1.63	2.63	2.71	2.02
	II	3.11	2.52	1.98	2.87	2.95
	III	2.13	1.79	1.93	3.40	3.13

TABLE 13

VARIANCE ANALYSIS OF THE DATA IN TABLE 12 FROM SHAM OPERATED ANIMALS

Main Factors	Degrees freedom	Sum squares	Variance	F	P
TREATMENT	4	7.7808	1.9452	7.296	0.01
SIDE	1	.4788	.4788	1.796	n.s.
EXPERIMENT	2	.1027	.0513	.193	n.s.
RESIDUAL	22	5.8656	.2666		

The interaction of all factors and the first order interactions were not significant. These sum of squares were therefore pooled with the residual sum of squares to obtain the estimate of residual variance.

TABLE 14

VARIANCE ANALYSIS OF DATA IN TABLE 12 FROM KINDLED RATS

	Degrees Freedom	Sum squares	Variance	F	P
<u>Main factors</u>					
TREATMENT	4	1.1116	.2779	1.472	n.s.
SIDE	1	5.8786	5.8786	52.291	0.001
EXPERIMENT	2	.8404	.4202	3.738	n.s.
TxS	4	1.1255	.2814	2.5028	n.s.
TxE	8	3.9306	.4913	4.370	0.05
SxE	2	.2929	.1465	1.303	n.s.
RESIDUAL	8	.8994	.1124		
Total	29	14.0790			

The Effect of Atropine on Kindling

From Table 15 and Table 16 it can be seen that the number of stimulations to kindling is not influenced by pretreatment with atropine either in a dose of 25 mg/kg or 2.5 mg/kg. Table 17 shows the results of the experiment to test the effect of atropine on after-discharge threshold and again atropine is without effect.

Note

In the first experiment (Table 15), testing the higher dose of atropine, the mean number of stimulations to kindle the 17 treated and control animals was 12.6 ± 4.3 (S.D.).

In the second experiment (Table 16), testing the lower dose of atropine, the mean number of stimulations for the 13 treated and control rats was 5.9 ± 2.3 .

The difference is highly significant ($P < .001$).

The two groups of rats used to test high and low dose atropine appeared to differ only in the time interval between operation and the start of stimulus. This delay could explain the highly significant difference in the rate of kindling observed, and these findings will be further discussed in chapter 3.

The Measurement of Muscarinic Receptor Numbers after Kindling in Rats

Saturability of specific QNB binding to rat brain preparations

The specific binding of QNB expressed as CPM is plotted against QNB concentration in Figure 11, which confirms that saturation was reached with a concentration of 5 mM QNB.

Muscarinic receptor numbers in kindling

Receptor numbers in the right and left amygdala, cortex, hypothalamus, hippocampus and reticular formation are shown in Table 18. No significant differences are found between right and left sides for each area or between kindled, sham-operated and unoperated groups.

The measurement of sodium-dependent choline uptake

Saturation of choline uptake

In Figure 12 it is shown that choline uptake saturates at concentrations of choline between 0.4 and 0.6 μ M. Further analysis of this data by means of a Lineweaver Burke plot (Table 19 and Figure 13). The K_m for the uptake is 0.4 μ moles and V_{max} is 7.6 p moles/4 Min./mg. protein.

TABLE 15

THE EFFECT OF ATROPINE 25 mg/kg ON THE RATE OF KINDLING
(Atropine sulphate or saline injected I.P. 1 hour before each stimulus)

ATROPINE TREATED			SALINE TREATED CONTROLS		
Rat	Number of Days From Operation To Start of Stimulations	Number of Stimulations Required to Kindle	Rat	Number of Days From Operation To Start of Stimulation	Number of Stimulations Required to Kindle
1	12	13	10	14	9
2	11	11	11	14	12
3	10	11	12	11	8
4	8	18	13	7	18
5	8	11	14	7	6
6	8	19	15	7	19
7	8	12	16	7	10
8	8	9	17	6	9
9	8	20			
Mean	9	13.8	Mean	9.1	11.4
SD	1.5	3.9	SD	3.1	4.4
N	9	9	N	8	8

The number of stimulations to kindle is not affected by Atropine 25 mg/kg (Wilcoxon's two sample Rank Test $p > .05$).

TABLE 16

THE EFFECT OF ATROPINE (2.5 mg/Kg) ON THE RATE OF KINDLING
(Atropine sulphate or saline injected I.P. 1 hour before each stimulus)

ATROPINE TREATED			SALINE TREATED CONTROLS		
Rat	Number of Days From Operation To Start of Stimulation	Number of Stimulations Required to Kindle	Rat	Number of Days From Operation To Start of Stimulation	Number of Stimulations Required to Kindle
18	33	7	25	33	3
19	33	8	26	33	5
20	33	7	27	33	7
21	33	8	28	32	6
22	25	1	29	32	6
23	25	10	30	25	4
24	25	5			
Mean	29.6	6.6	Mean	31.3	5.2
SD	4.0	2.7	SD	2.9	1.3
N	7	7	N	6	6

The number of stimulations to kindle is not affected by Atropine 2.5 mg/Kg (Wilcoxon's two sample Rank Test $p > .05$ two tailed).

TABLE 17

THE EFFECT OF TWO DIFFERENT DOSES OF ATROPINE ON AFTER-
DISCHARGE THRESHOLD

After-discharge Threshold (μ amps)

<u>Rat</u>	<u>Day 1</u> <u>Saline</u>	<u>Day 2</u> <u>Saline</u>	<u>Day 3</u> <u>Saline</u>	<u>Day 4</u> <u>Atropine</u> <u>25 mg/Kg</u>	<u>Day 5</u> <u>Saline</u>	<u>Day 6</u> <u>Atropine</u> <u>2.5 mg/Kg</u>	<u>Day 7</u> <u>Saline</u>
1	-	100	100	140	120	80	120
2	80	60	80	80	60	60	80
3	80	80	120	120	80	60	80
5	240	240	220	220	220	220	220
8	100	120	100	80	80	80	80
10	160	160	160	160			
16	160	200	220	200	240	240	200

These results show that atropine does not influence the after-discharge threshold in these kindled rats.

(Wilcoxon signed rank test $P > .05$).

Saturation of specific QNB binding in rat cortex

FIGURE II

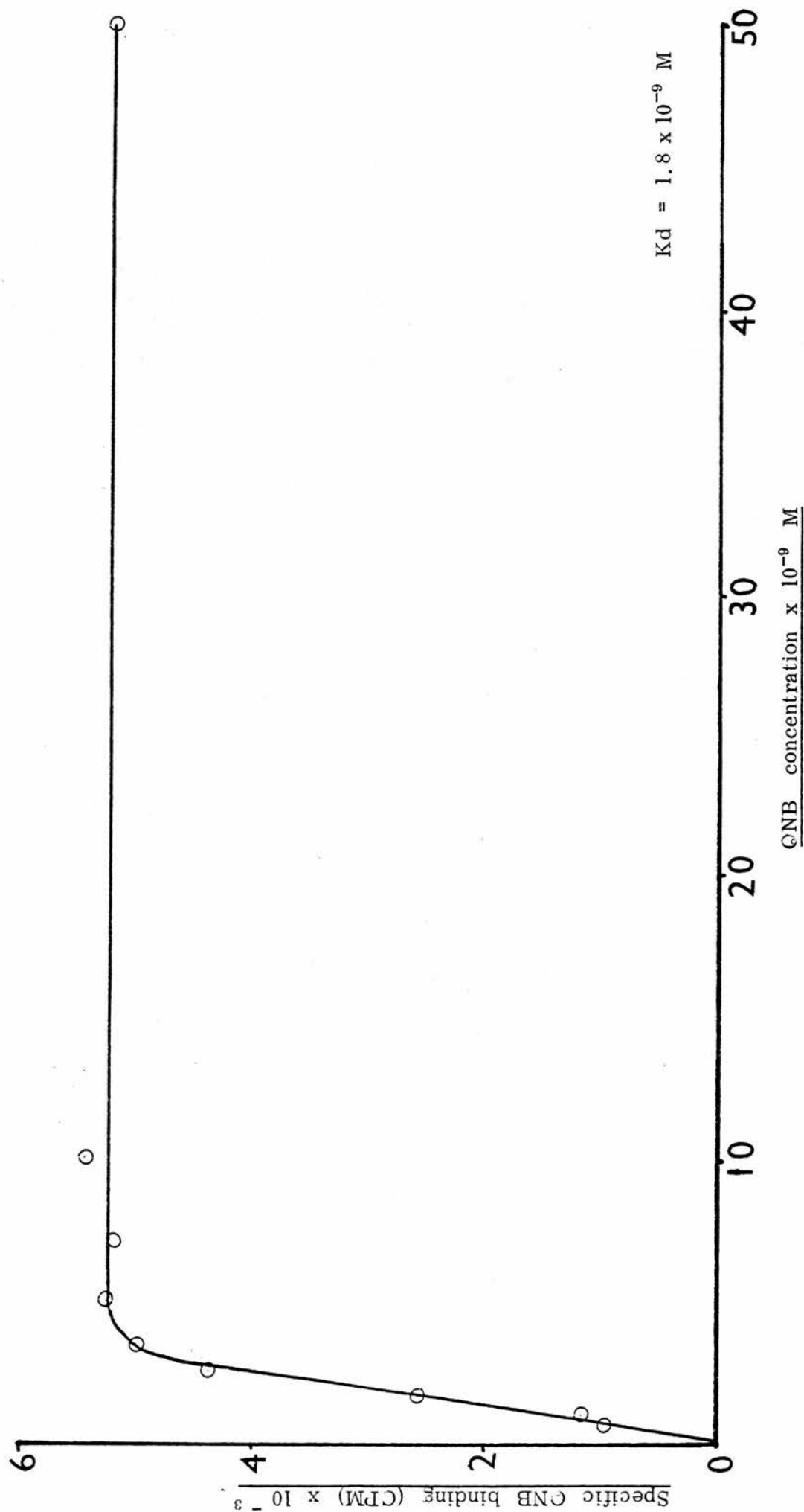


TABLE 18

THE EFFECT OF KINDLING ON
MUSCARINIC RECEPTOR NUMBERS
IN VARIOUS REGIONS OF THE RAT BRAIN
(p moles QNB/g Protein)
Mean \pm SEM (N)

Brain Region	Kindled	Sham Operated Controls	Unoperated Controls
RIGHT AMYGDALA	445 \pm 19.6 (10)	470 \pm 31 (13)	600 \pm 68.4 (5)
LEFT AMYGDALA	460 \pm 31.6 (10)	472 \pm 22.7 (13)	518 \pm 53.4 (5)
RIGHT CORTEX	427 \pm 52.8 (5)	586.6 \pm 66.6 (5)	
LEFT CORTEX	501 \pm 103.6 (5)	530.6 \pm 24.3 (5)	

TABLE 18 (continued)

THE EFFECT OF KINDLING ON
MUSCARINIC RECEPTOR NUMBERS
IN VARIOUS REGIONS OF THE RAT BRAIN
(p moles QNB/g Protein)
Mean \pm SEM (N)

Brain Region	Kindled	Sham Operated Controls	Unoperated Controls
RIGHT HYPOTHALAMUS	307 \pm 13.4 (5)	322 \pm 17.4 (5)	
LEFT HYPOTHALAMUS	308 \pm 23.3 (5)	301 \pm 21.0 (5)	
RIGHT HIPPOCAMPUS	560 \pm 8. (5)	590 \pm 16.1 (5)	
LEFT HIPPOCAMPUS	593 \pm 44.7 (5)	590 \pm 58.1 (5)	
R. RETICULAR FORMATION	316 \pm 46.9 (5)	400 \pm 25.9 (5)	
L. RETICULAR FORMATION	362 \pm 33.5 (5)	350 \pm 29.9 (5)	

The differences between right and left sides and between kindled, sham operated and unoperated control groups is not significant (p > .05 Student t test).

FIGURE 12

SATURATION OF HIGH AFFINITY
SODIUM DEPENDENT CHOLINE
UPTAKE

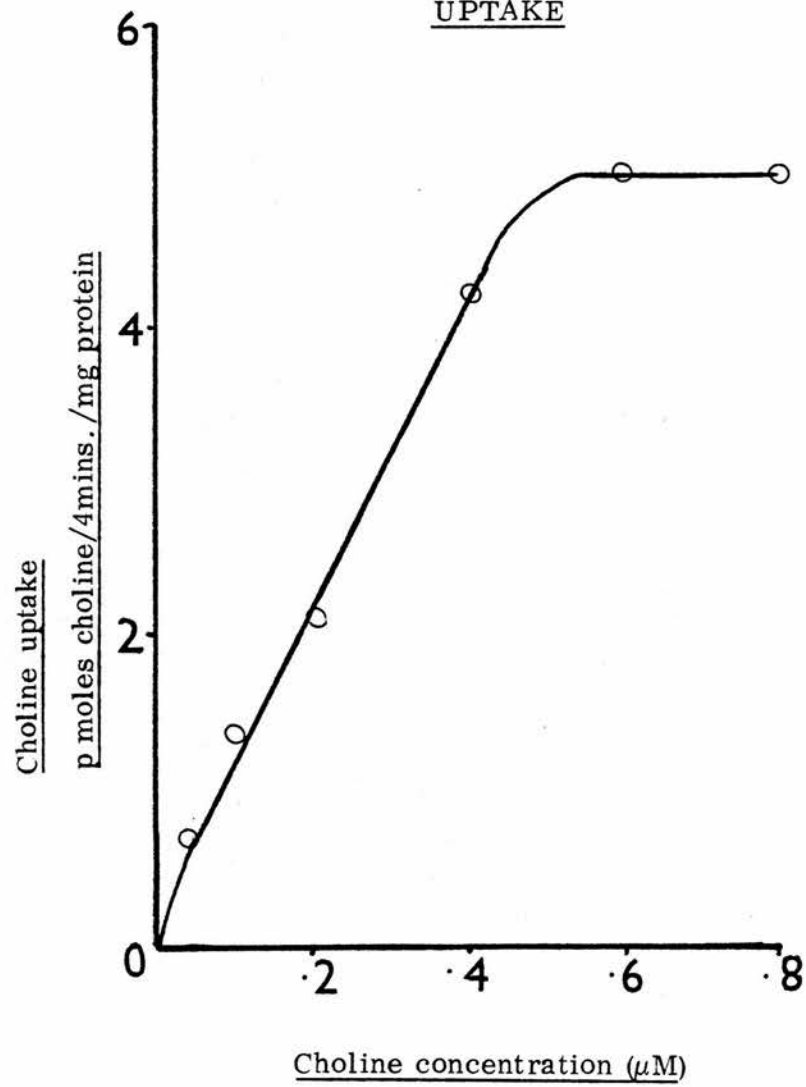


TABLE 19

CHOLINE UPTAKEDATA FOR LINEWEAVER-BURKE PLOT

(from Figure 12)

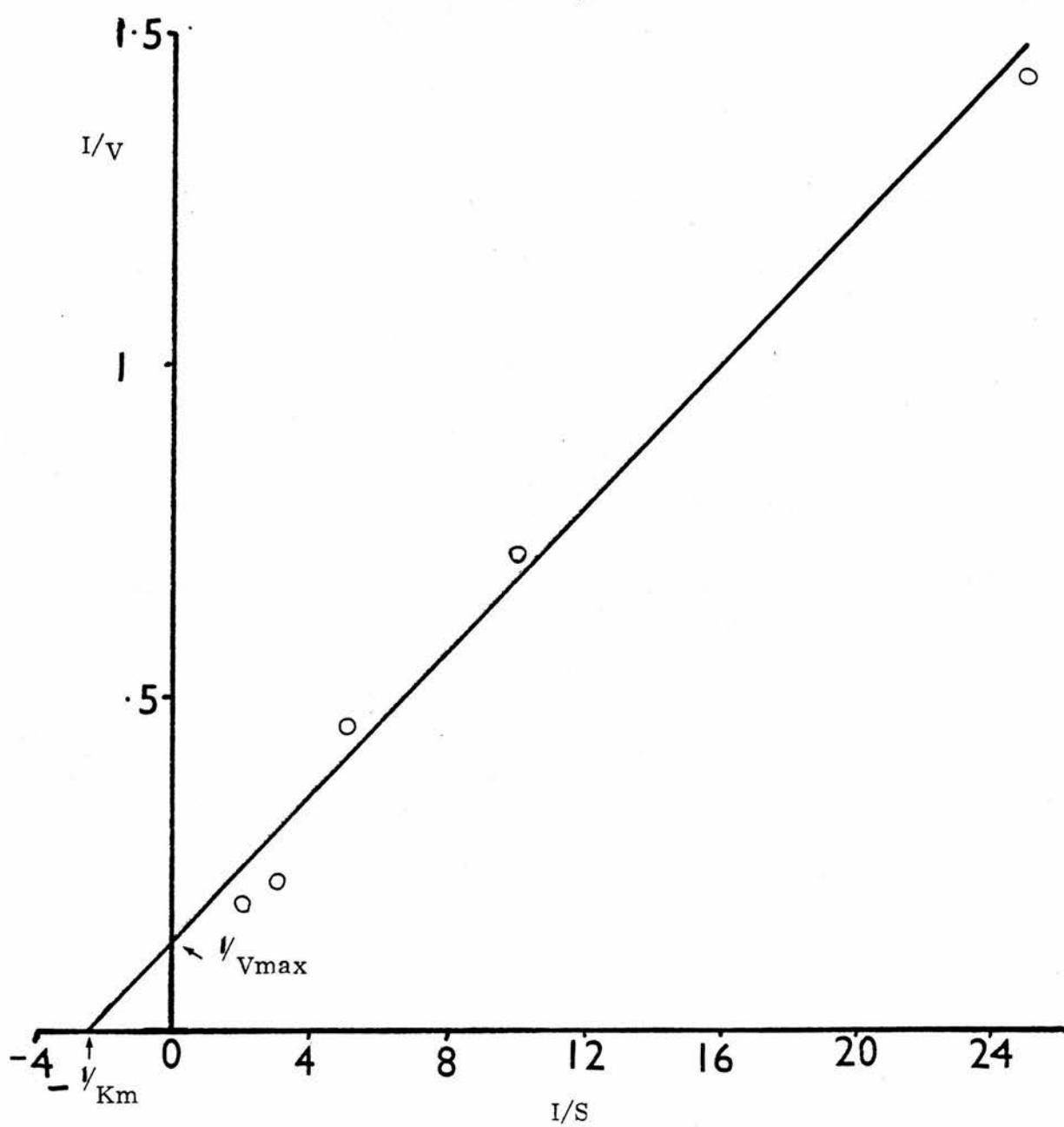
<u>V (uptake)</u>	<u>1/V</u>	<u>S</u>	<u>1/S</u>
0.7	1.43	.04	25
1.4	0.74	.1	10
2.2	0.46	.2	5
4.5	0.22	.4	2.5
5.1	0.2	.6	2
5.0	0.2	.8	1.25

Calculation of K_m and V_{max} Correlation coefficient $r = 0.995$ ($\pm .006$) S.E.M. $a = 0.1332$ $b = 0.053$ For $y = a + bx$ Thence: $K_m = 0.4 \mu\text{Moles}$ $V_{max} = 7.6 \text{ pMoles/4 min./mg protein}$

FIGURE 13

HIGH AFFINITY SODIUM DEPENDENT
CHOLINE UPTAKE

Lineweaver-Burk plot of data in
Table (19)



The Effect of Protein Concentration on the Assay of Choline Uptake

Figure 14 shows that protein in excess of 0.36 mg. in the reaction mixture interferes with the measurement of high affinity sodium dependent choline uptake. In all the experiments reported 100 μ l of brain homogenate contained about 0.12 mg. protein which was well below the critical level.

The Effect of Pentobarbitone Treatment on Choline Uptake

The results shown in Table 19a demonstrate that pentobarbitone pretreatment causes a 53% reduction in choline uptake subsequently measured in hippocampal homogenates. This confirms that this assay is capable of detecting activity related changes in choline uptake.

Choline Uptake in Regions of the Brain of Kindled, Sham-operated and Unoperated rats

Table 20 gives the result of the estimation of choline uptake in the right and left amygdala of kindled, sham and unoperated rats and the right and left hippocampus, striatum and cortex of kindled and sham rats.

Differences between right and left sides and between the same regions in different groups were not significant.

Measurement of Acetylcholinesterase in Kindling

In Table 21 are shown the results of the assay of acetylcholinesterase in the right and left amygdala, cortex and striatum of kindled, sham and unoperated rats.

Kindling had no effect on the enzyme activity.

ATPase in Kindling

Table 22 contains the measured activities of total ATPase and Mg dependent ATPase in the amygdala, cortex and striatum of kindled and sham operated rats. Kindling has no effect on ATPase activity.

FIGURE 14

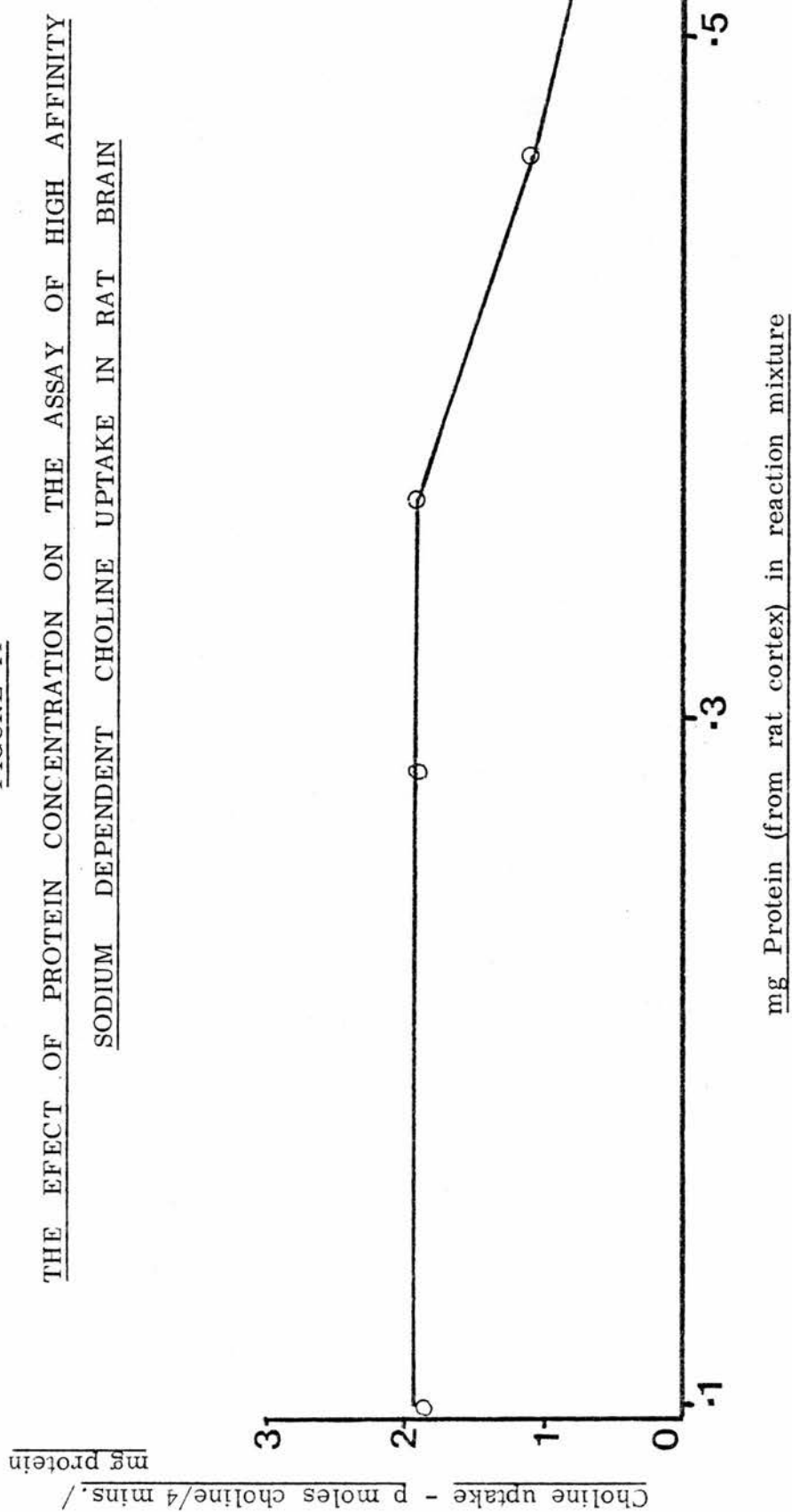


TABLE 19a

ACTIVITY RELATED CHANGE IN CHOLINE UPTAKE. CONTROLS
RECEIVED 0.5 ml. SALINE I. P. PENTOBARBITONE TREATED
ANIMALS RECEIVED 65 mg/Kg 30 MINUTES BEFORE DECAPITATION

High Affinity Choline Uptake (CPM/mg Protein)

<u>Saline treated</u>		<u>Pentobarbitonetreated</u>	
	1.14		0.34
	0.98		0.58
	0.89		0.62
	1.08		0.39
\bar{x}	1.02		0.48
SD	0.095		0.108
N	4		5

$$t = 7.043$$

$$P < .001 \text{ (two tail)}$$

Reduction in choline uptake is 53%.

SODIUM-DEPENDENT HIGH AFFINITY CHOLINE UPTAKE (p MOLES/
4 MINUTES/mg PROTEIN) IN VARIOUS REGIONS OF RAT BRAIN IN
KINDLED, SHAM-OPERATED AND UNOPERATED RATS

	<u>R. Amygdala</u>			<u>L. Amygdala</u>		
	<u>Kindled</u>	<u>Sham</u>	<u>Unoperated</u>	<u>Kindled</u>	<u>Sham</u>	<u>Unoperated</u>
Mean	17	15.2	15.1	16.8	14.5	16.8
S. D.	2.3	4.8	5.8	4	3.4	
N	6	6	8	6	6	8
	<u>R. Hippocampus</u>		<u>L. Hippocampus</u>			
	<u>Kindled</u>	<u>Sham</u>	<u>Kindled</u>	<u>Sham</u>		
Mean	12.9	12.8	12.9	12.3		
S. D.	2.0	2.7	1.6	2.6		
N	7	5	6	5		
	<u>R. Striatum</u>		<u>L. Striatum</u>			
	<u>Kindled</u>	<u>Sham</u>	<u>Kindled</u>	<u>Sham</u>		
Mean	32.0	28.3	31.5	28.3		
S. D.	4.7	6.3	4.3	7.6		
N	6	5	6	5		
	<u>R. Cortex</u>		<u>L. Cortex</u>			
	<u>Kindled</u>	<u>Sham</u>	<u>Kindled</u>	<u>Sham</u>		
Mean	7.7	7.6	8.2	7.8		
S. D.	0.8	1.8	0.9	2.0		
N	6	5	6	5		

The differences between right and left and between kindled, sham-operated and unoperated are not significant ($P > .05$ student t test).

THE EFFECT OF KINDLING ON ACETYLCHOLINE ESTERASE ACTIVITY
(μ MOLES/HOUR/g WET WEIGHT) IN VARIOUS REGIONS OF RAT BRAIN

MEAN \pm SEM (N)

<u>Brain Region</u>	<u>Kindled</u>	<u>Sham</u>	<u>Unoperated</u>
Right Amygdala	911 \pm 96.5 (5)	1050 \pm 74.2 (6)	1110 \pm 97 (4)
Left Amygdala	986 \pm 74.4 (5)	958 \pm 61.5 (6)	1030 \pm 72 (4)
Right Cortex	595 \pm 114 (4)	757 \pm 75.8 (6)	580 \pm 33.3 (4)
Left Cortex	610 \pm 50.3 (4)	712 \pm 96.3 (6)	678 \pm 89.1 (4)
Right Striatum	1093 \pm 84.2 (5)	1077 \pm 79.1 (6)	1116 \pm 85.2 (4)
Left Striatum	1073 \pm 54.1 (5)	892 \pm 130 (6)	1147 \pm 68.8 (4)

Differences between right and left sides and between kindled, sham and unoperated groups are not significant at the 5% level (student t test).

TABLE 22

THE EFFECT OF KINDLING ON THE ACTIVITY
OF ADENOSINE TRIPHOSPHATASE
(μ Moles Phosphate/Hour/mg Protein)
in various regions of the rat brain
MEAN \pm SEM

	Total ATPase		Mg ATPase (Oubain Insensitive)		Na/K ATPase (Oubain Sensitive)	
	Kindled N = 6	Sham N = 6	Kindled N = 6	Sham N = 6	Kindled N = 6	Sham N = 6
R. AMYGDALA	4.2 \pm .5	4.3 \pm .7	3.0 \pm .5	2.8 \pm .5	1.2 \pm .4	1.5 \pm .3
L. AMYGDALA	5.3 \pm .4	5.6 \pm .8	3.9 \pm .4	3.4 \pm .4	1.3 \pm .4	2.2 \pm .6
R. CORTEX	6.0 \pm .7	6.1 \pm .6	3.7 \pm .3	3.3 \pm .3	2.8 \pm .6	2.8 \pm .3
L. CORTEX	6.0 \pm .7	5.8 \pm .5	3.4 \pm .9	3.0 \pm .4	1.9 \pm .1	2.5 \pm .5

TABLE 22 (continued)

THE EFFECT OF KINDLING ON THE ACTIVITY
OF ADENOSINE TRIPHOSPHATASE
(μ Moles Phosphate/Hour/mg Protein)
in various regions of the rat brain
MEAN \pm SEM

	Total ATPase		Mg ATPase (Oubain Insensitive)		Na/K ATPase (Oubain Sensitive)	
	Kindled N = 6	Sham N = 6	Kindled N = 6	Sham N = 6	Kindled N = 6	Sham N = 6
R. STRIATUM	7.1 \pm .2	6.6 \pm .4	4.8 \pm .3	4.4 \pm .3	2.3 \pm .2	2.2 \pm .5
L. STRIATUM	6.4 \pm .5	6.7 \pm .6	4.6 \pm .3	4.3 \pm .2	1.7 \pm .3	2.3 \pm .3

The differences between right and left sides and between kindled and sham-operated groups are not significant in any brain region ($p > .05$ student t test).

2 - 4 DISCUSSION OF RESULTS(a) Noradrenaline and Dopamine in KindlingNoradrenaline

The basal concentration of noradrenaline was significantly lower in the left amygdala compared to the right in kindled animals. However, it should be noted also that the concentration in the left amygdala did not differ from the left or right amygdala in the sham-operated or the unoperated groups.

Noradrenaline turnover rates did not differ when right and left sides were compared in the amygdala or hippocampus and there were no significant differences between kindled, sham-operated and unoperated groups. Since the turnover rate of catecholamines is probably a better index of activity than the concentration of neurotransmitter, it can be concluded that these results fail to show an effect on noradrenaline in the amygdala following kindling.

Dopamine

~~Although~~ The basal concentration of dopamine did not differ significantly between right and left sides in kindled, sham-operated or unoperated controls. The rate of dopamine turnover was increased in the left amygdala compared to the right amygdala (Table 10). This change was not observed in the hippocampus. The significance of the finding is however doubtful in view of the equality of all other contrasts (in Table 10).

Comparison of turnover kinetics of catecholamines with data published

The turnover rates calculated for noradrenaline (Table 4) and dopamine (Table 5) in the rat amygdala are in reasonably close agreement with the results obtained by Brodie et al (1966), although direct comparison is not possible as these authors studied whole rat brain.

They found quite wide variation of results between two strains of rats. For noradrenaline the rates of synthesis were 0.036 and 0.071 $\mu\text{g/g/hour}$. The turnover times were 8.5 and 5.8 hours and the rate constants 0.12 and 0.17.

For dopamine the rates of synthesis were 0.21 and 0.43 $\mu\text{g/hour/g}$. The turnover times 3.6 hours and 2.7 hours and the rate constants 0.28 and 0.39.

Conclusion

Although a central role for noradrenaline in kindling is strongly suggested

by the pharmacological evidence outline in section 1 - 9, adequate biochemical evidence for an amine involvement in kindling is still lacking. The demonstration of reduced β -adrenergic receptor numbers in the right and left amygdala (McNamara, 1978b) and our finding²⁰¹¹ of a reduced level of tyrosine hydroxylase in the stimulated amygdala are in keeping with an alteration in noradrenaline function. However, I have been unable to measure a long-term alteration in the turnover kinetics of noradrenaline in the amygdala or hippocampus in kindled rats.

My experiments on dopamine turnover are inconclusive but an increase in dopamine turnover rate in the stimulated amygdala one month after kindling would be consistent with

the demonstration by Gee et al (1979) of changes in dopaminergic receptors as measured by the radioligand ^3H -spiroperidol and antagonist (+) butaclamol in the amygdalae of kindled rats. These authors reported a reduction in both number and affinity of binding sites in the left and right amygdala after kindling, reflecting a dopaminergic subsensitivity. Enhanced release of dopamine or availability of agonist has been reported to cause dopamine receptor subsensitivity in other areas of the central nervous system (Quirk and Iversen, 1978).

However, in the experiments of Gee et al (1979) rats were killed only 24 hours after a convulsion and the changes could have been unrelated to kindling because β receptors are reduced following electroshock (Bergstrom and Kellar, 1979).

Secondly, there is some doubt about the specificity of this binding assay which may detect 5-HT and dopamine receptors (Leysen et al, 1979).

Finally, the statistical handling of their data by these authors is puzzling, since having performed five assays using material pooled from 20 animals, they appear to have calculated standard errors of the means on the basis of $N = 20$, rather than $N = 5$.

Other biochemical evidence that dopamine is involved in kindling is not compelling, and furthermore specific dopaminergic agonists L-dopa and bromocriptine have no effect on kindling (Farjo, 1978). Engel and Sharpless (1977) reported a lasting reduction in dopamine concentration measured one month after the cessation of kindling. However, their control group of rats appears to have been selected in retrospect and to have been far from uni-

form, and included rats which were stimulated but had failed to kindle. Nevertheless, the difference in dopamine concentration was significantly lower in the left stimulated amygdala compared to the right side in kindled rats.

In general, amine tissue concentration is not an accurate reflection of catecholamine function and Brodie et al (1966) showed that amine tissue concentration may remain constant or even decline despite an increased rate of synthesis. Several published reports purporting to demonstrate changes in monoamine levels after kindling only obfuscate the issue (compare Callaghan and Schwarz, 1976 with Callaghan and Schwarz, 1979:- two papers which give completely different findings without any comment in the second paper about the contrary results given in the first).

Despite the shortcomings of the biochemical evidence, it remains a reasonable hypothesis that noradrenaline is involved in the development of kindling and dopamine is a reasonable candidate for a role in the long-term changes controlling cell excitability.

Our knowledge of the role of catecholamines, particularly noradrenaline, in other long-term adaptive processes lends weight to the argument.

The transynaptic regulation of the synthesis of the enzyme tyrosine hydroxylase and dopamine β -hydroxylase has been proposed as an important mechanism for mediating long-term adaptive processes in the central nervous system (Thoenen and Otten, 1977) and it is tempting to speculate that a similar type of adaptive process could underlie the enduring effect of kindling.

It is well established that electrical stimulation of the locus coeruleus leads to the induction of synthesis of new molecules of TOH and DBH and that after a delay of several days the newly synthesized enzyme is transported to nerve terminals in limbic structures (Zigmond, 1979).

Ross et al (1975) have also demonstrated that reduced neurotransmission may be accompanied by a reduction in tyrosine hydroxylase and dopamine β -hydroxylase activity. After transection of the efferent noradrenergic fibres from locus coeruleus in the medial forebrain bundle there was a reversible loss of tyrosine hydroxylase and dopamine β -hydroxylase in the cell body and all collateral fibres, suggesting that the reduced enzyme synthesis reflects the reduced requirements for noradrenergic neurotransmission in the cell.

If a parallel is made with kindling, it could be proposed that the reduction in tyrosine hydroxylase, β receptors and noradrenaline levels reflect long-term reduced activity in the noradrenergic innervation of the amygdala which originates in locus coeruleus and brain stem and this reduced noradrenaline activity is associated with seizures.

It is noteworthy that repeated stimulation of the locus coeruleus does not produce convulsions (Phillips, 1976) and leads to TOH induction which can be measured in limbic structures (Zigmond, 1979), whereas stimulation of areas to which noradrenergic fibres project, such as the amygdala, causes a reduction in TOH (Fuxe and Gunne, 1964) and kindled convulsions. In the peripheral sympathetic system Thoenen and Otten (1977) have shown that acetylcholine is the effector for the stimulus evoked induction of tyrosine hydroxylase. The mechanism whereby kindling stimulation in the amygdala could modulate TOH synthesis in the brain stem noradrenergic nuclei is entirely unknown.

(b) The Role of c GMP in Kindling

The animals used in the experiment were killed three months after their last convulsion. They could be assumed to be still in a kindled state, since previously with similar rats I had evoked generalized convulsions after one or two stimulations ten months after kindling.

The increase in basal level of cyclic GMP in the kindled amygdala is in accord with the finding of increased cyclic GMP in all phases of focal epilepsy induced by penicillin in the cat cortex (Palmer et al, 1976). These authors found an increase in cyclic GMP not only in periods of neuronal excitation during a convulsion, but also during the period of postictal depression, suggesting that the role of cyclic GMP in epilepsy is not limited to the time of actual fitting.

There is no clear explanation for the significant difference between left and right sides in the kindled amygdala (Tables 12 and 13). However, it is of interest that the changes in cyclic GMP do occur on the unstimulated (right) side because it has been shown electrographically that the contralateral amygdala is involved early in the development of kindling and in fully kindled animals recordings show equal activity in both amygdalae (Racine, 1972b).

I have used similar incubation conditions to Ferrendelli et al (1972b), who reported an increase in cyclic GMP levels in brain slices incubated with depolarizing concentrations of K^+ and a smaller increase in the presence of Ca^{++} alone. The depolarization-induced elevation of cyclic GMP was Ca^{++} dependent and the demonstration that the effect of divalent cations on cyclic GMP was parallel to their effect on neurotransmitter release supported the hypothesis that cyclic GMP accumulation is linked to the process of neurotransmitter release. In contrast to the depolarization induced increase in cyclic GMP which is observed in all brain areas, there is evidence for regional differences in the response of cyclic GMP to neurotransmitters, since noradrenaline is effective in producing a rise of cyclic GMP in cerebellar slices, but not in other brain regions (Ferrendelli, 1976). Results from sham-operated rats show that dopamine causes an increase in cyclic GMP in amygdala slices and this contrasts with a reduction in the cerebellum (Ferrendelli, 1976).

It is possible that the increase in the basal level of cyclic GMP and the impaired response to depolarization, dopamine and haloperidol in kindling reflects altered neurotransmitter release. However, further knowledge is required about the location and compartmentation of the cyclic GMP for this to be confirmed.

(c) Studies on the Cholinergic System after Kindling

Effect of atropine

Atropine in two different doses had no effect on the rate of kindling nor on the after-discharge threshold.

Different experiments have found inconsistent effects of atropine on amygdaloid kindling in rats (Section 1 - 9). My own findings are in agreement with those of Corcoran et al (1976) and in disagreement with Arnold et al (1973) and Albright et al (1979).

These latter authors ruled out a species effect. One possible explanation is that, since atropine is known to desynchronize the EEG by an effect on the ascending reticular activating system, stress could be amplified in atropine treated rats. When kindling is delayed by atropine it could be due to increased stress in some laboratories due to difference in animal welfare and handling.

Muscarinic Receptor Number (Table 18)

No change was measured in the right and left amygdala, cortex, hypothalamus, hippocampus or reticular formation. Furthermore, the results obtained for cortex were in broad agreement with the reported findings of other workers who have quoted levels in the range of 1.7 ± 0.5 nmol QNB/g protein in synaptosome prepared from rat cortex (Birdsall et al, 1976, Hulme et al, 1976). Taking into account the 3 - 4 fold 'purification' of protein during synaptosome preparation from crude brain homogenates, the level of 0.5 nmols QNB/g protein in a crude brain homogenate from rat cortex, shown in Table 18, is within the same range.

This data is in agreement with McNamara (1978), who also found no lasting change in the number of muscarinic receptors in the amygdala of kindled rats measured 3 days after a convulsion. The report by this author that muscarinic receptor numbers are reduced when measured 15 hours after a kindled seizure could be an outcome of seizures themselves, since an increase in cholinergic activity is known to follow spontaneous seizures or single or repeated electroshock (Green et al, 1979) and increased acetylcholine release could lead to a compensatory reduction in receptor numbers.

High Affinity Choline Uptake

The results from kindled and sham-operated rats did not differ significantly from unoperated controls in the right and left amygdala, hippocampus, striatum or cortex.

It has been confirmed that this assay is reliable and will detect a 53% reduction in choline uptake in the hippocampus following barbiturate administration (Table 19a). Therefore, the assay procedure using a debris-free brain homogenate yields equivalent results to other workers'.

The results in Table 20 cannot be compared directly with those published by Simon et al (1976), since these authors measured rat brain choline uptake in synaptosome fractions (P_2) at 30°C using $0.54 \mu\text{M}$ ^3H -choline. I have measured uptake in crude debris-free homogenates (P_1) using $0.6 \mu\text{M}$ ^3H -choline. The loss of protein when a P_1 fraction was centrifuged at high speed to prepare a P_2 synaptosomal fraction was in the range 34 - 46%.

When allowance is made for the purification of protein our results are in reasonable agreement. Simon et al (1976) quote the following rates of

choline uptake (p moles/4 min./mg. protein).

Cortex 16.1 Hippocampus 21.4 Striatum 69

They measured a K_m of $0.3 \mu M$ and V_{max} of 25 p moles/4 min./mg. protein (cf. Table 19).

There is good evidence that measurement of the sodium dependent high affinity choline transport system in brain tissue in vitro is a reliable measure of the functional activity of cholinergic neurones in vivo (Atweh et al, 1976).

Treatments which alter the activity of cholinergic neurones cause parallel changes in uptake. Thus reduced cholinergic activity in the hippocampus following the administration of anaesthetics or the section of the septo-hippocampal path can be measured in vitro as a reduced synaptosomal uptake. The converse is found following electrical stimulation of the septo-hippocampal pathway, or the administration of the convulsant pentylenetetrazol, both of which treatments cause an increase in synaptosomal choline uptake. The changes occur in V_{max} and not the affinity of the uptake system.

Acetylcholinesterase

Activity of this enzyme was not altered in kindling in the right and left amygdala, cortex or striatum.

Conclusion

The findings that choline uptake is unaltered in several brain regions, including the amygdala, one month after kindling, taken together with the absence of change in muscarinic receptor numbers, is a strong indication that a permanent change in cholinergic function does not occur in kindling.

It is not possible to rule out a role for cholinergic systems during kindling development, but it would require a very full understanding of seizure mechanisms to rule out non-specific changes associated only with ictus.

(d) Changes in Membranes in Kindling

Clearly neurotransmitter related changes are only one of several types of process which could be taking place in kindling.

By measuring both ouabain-sensitive and ouabain-insensitive ATPase **I** have shown that kindling is not accompanied by an alteration in ion pump

activity nor by changes in ATPases which are believed to be altered in some form of reflex epilepsy (Section 2-1).

Robinson et al (1975) showed that glia cell ATPase was elevated from up to 60 days after electrode implantation in the hippocampus. Therefore, the measurement of normal ATPase concentrations and to a lesser extent the normal ACE activities support the notion that kindling is not accompanied by widespread damage to neurones on glia at the stimulus site.

CHAPTER 3

THE EFFECT OF THE TIME LAPSE BETWEEN IMPLANTATION OF
ELECTRODES AND THE START OF KINDLING ON THE RATE OF KIND-
LING FROM THE RAT AMYGDALA

3 - 1 METHODS

Electrodes were constructed from stainless steel or platinum/iridium wire, as described in section 2 - 2. After operation, animals were housed in groups of four and managed in the same way as described for other kindling experiments, except that stimulation was administered on seven days a week, twice daily (between 10 a.m. and 12 a.m. and between 3 p.m. and 5 p.m.) with an interval of at least four hours between stimuli. A current intensity of 300 μ a was always used so the stimulus parameters did not vary between groups. Animals were withdrawn from the experiment if:

1. the electrodes were short circuited,
2. after-discharge could be evoked only by a stimulus current greater than 300 μ a,
3. animals had not kindled after 40 stimulations,
4. obvious stress accompanied stimulus (humanitarian considerations apart, previous experience had shown that obvious stress was not a feature of normal amygdaloid kindling).

My experience over three years was that about 70% of operated animals kindled successfully.

Kindling as usual was considered complete when a generalized seizure followed three consecutive stimulations.

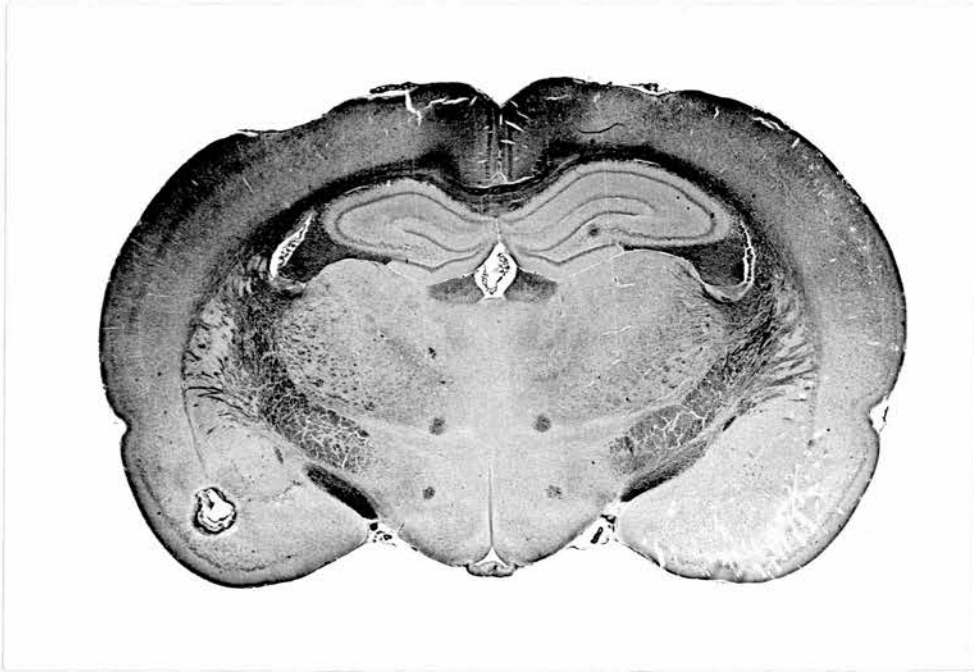
Electrode position was confirmed histologically using either frozen or wax-embedded sections. Frozen sections were stained using toluidene blue and wax-embedded sections were stained using luxol fast blue and counter-stained with cresyl violet (Figure 15).

Histological Verification of the Position of Electrodes

Lesion making and dissection of the brain

Animals were anaesthetized with pentobarbitone sodium ('Sagatal', May and Baker) 0.4 mls./kg I.P. and a lesion was created at the elec-

FIGURE 15



Section of rat brain showing the position of a lesion made at the electrode tip in the amygdala on the left of the photograph (Luxol fast blue and cresyl violet stain).

trode tip by the passage of 6 m coulombs to the bipolar electrode from a Farnell physiological stimulator. After 5 minutes, the animal was killed by a blow on the thorax, decapitated and the brain removed to ice-cold saline before dissection into three parts by two vertical cuts at the level of the optic chiasm and emergence of the cerebral peduncles posteriorly.

The middle third of the brain contained the electrode site. Subsequent histology was performed either on wax-embedded sections or frozen cryostat sections.

Preparation of wax-embedded sections

The brain sections were fixed and dehydrated in a Histokinette (Hendley Relays, Slough, Bucks.) with the following schedule:

Bouin's fixative	6 hours
50% ethanol	2 hours
70% "	1 hour
90% "	1 hour
95% "	1 hour
95% "	1 hour
100% "	1 hour
100% "	1 hour
100% "	1 hour
100% "	1 hour
Toluene	1 hour
Toluene	1 hour
<u>Total</u>	18 hours

After removal from the histokinette, brain sections were embedded in paraffin wax (congealing point 54.5°C B.D.H.) using a vacuum embedding oven (Hearson, Willow Walk, London) at a pressure of -250 mm. H_g and a temperature of 60°C. Tissue was placed in a beaker of wax for 60 minutes with one change of wax to clean off excess toluene. After cooling, the wax blocks could be stored indefinitely.

8 μ sections were cut by microtomy (Leitz GMBH Wetzlar - Minot - Mikrotom Type 1212), floated on water at 44°C in a humid atmosphere, and mounted on gelatin coated slides. slides were thoroughly dried in an oven

at 40°C overnight, as this was necessary to prevent dislodgement of the sections.

Preparation of gelatin-coated slides

A solution was prepared containing 2 g. gelatin and 0.2 g. chromic potassium sulphate in 400 ml. water. Clean microscope slides in stainless steel racks were dipped into the coating solution and allowed to dry overnight at room temperature before use.

Staining

Before staining, ^{EXCESS WAX WAS REMOVED} by overnight immersion in xylene in a staining dish, and subsequently placed in 100% ethanol to wash off the xylene.

Luxol fast blue method for staining myelin with cresyl violet counter-stain for grey matter

(Carleton's Histological Technique, 1967)

The method was modified slightly. After ^{REMOVAL OF EXCESS WAX} the glass slides held in stainless steel racks were immersed in 95% ethanol for 30 minutes followed by luxol fast blue solution for 30 minutes at room temperature. (Luxol fast blue solution was prepared by dissolving 0.1 g. of the dye in 0.5 ml. 10% ethanol before adding 100 ml. 95% ethanol). The slides were thoroughly rinsed in 95% ethanol before differentiation of the stain using 0.05% lithium carbonate and 70% ethanol. Slides were dipped for about 5 seconds into a 0.05% lithium carbonate solution, agitated for about 30 seconds in 70% ethanol, before being rinsed thoroughly in distilled water for at least 2 minutes. The differentiation sequence was repeated until to naked eye inspection the grey matter appeared colourless and only myelin remained stained. The slides were again washed in distilled water for 10 minutes to remove all trace of lithium carbonate before proceeding to counter-stain the grey matter using cresyl fast violet (CFV) (Fluka A.G. Buchs 56 Swizerland).

Slides were dipped into a solution of 0.1% CFV in distilled water for 4 minutes at room temperature, rinsed in distilled water and differentiated in 95% ethanol for about 5 minutes or until the colour appeared right.

Finally, the tissue was dehydrated in 100% ethanol for 5 minutes, placed in xylene for 5 minutes and mounted using DPX mountant.

Alternatively, for more rapid results, 10μ - 40μ frozen cryostat sections were stained using toluidene blue.

Preparation of frozen sections

The dissected brain section was placed on a small circular piece of cork 2 cm. diameter and 3 mm. thick (about $\frac{1}{3}$ of each brain on a cork).

The cork was placed on a cryostat chuck with a water drop lying between the metal and cork surface, so that the brain, cork and chuck could be rapidly frozen using a carbon dioxide bench freezing unit (SCEE, London) and transferred to a cryostat (Linde, West Germany) at a temperature of -25°C . After 30 minutes to equilibrate to the temperature of the cryostat the brain section was cut into $10 - 40\mu$ slices, which were laid out on a gelatin-chrome alum coated glass slide and made to adhere to the slide by thawing with the warmth of a finger pressed against the underside of the glass. Tissue sections mounted in this way could be stained immediately by the toluidene blue stain.

Toluidene blue staining of brain tissue sections

Ref. Laszlo (1977)

10μ or 40μ sections on microscope slides were placed in a coplin jar containing 2 ml. of formalin for fixation by formalin vapour. After 1 minute, the slides were placed over glass rods which bridged a staining trough. Sections were treated with the following solutions, applied by dropper, before being mounted on Canada balsam with clean glass cover slips for light microscopic observation.

- | | | |
|----|---------------------|-----------------|
| 1. | 100% ethanol | 10 - 20 seconds |
| 2. | 50% ethanol | 10 - 20 seconds |
| 3. | Distilled water | 10 - 20 seconds |
| 4. | 0.2% toluidene blue | 30 seconds |
| 5. | 100% ethanol | 10 - 20 seconds |
| 6. | Xylene | |

3 - 2 RESULTS

Table 23 shows the number of stimulations required to kindle rats approximately one week and one month after the implantation of either stainless steel or platinum/iridium electrodes.

At both of the times tested there was no significant difference between the performance of steel or platinum, which confirms Goddard's original observation (1969). However, with both types of metal rats kindled significantly faster after the longer time interval from operation. This difference was significant ($P < .05$ for stainless steel and $P < .02$ for platinum). These experiments confirm that the difference in kindling rates observed between the two groups of rats used in the atropine experiments (Section 2-3) could well have been due to longer implantation times, although in that instance the effect was much more marked (Tables 15 and 16).

The Placement of Electrodes

Figure 16 depicts the electrode position in 21 rats which were implanted with stainless steel electrodes.

Figure 17 illustrates the anatomical site of the tip of platinum/iridium electrodes in the animals on which Table 23 is based.

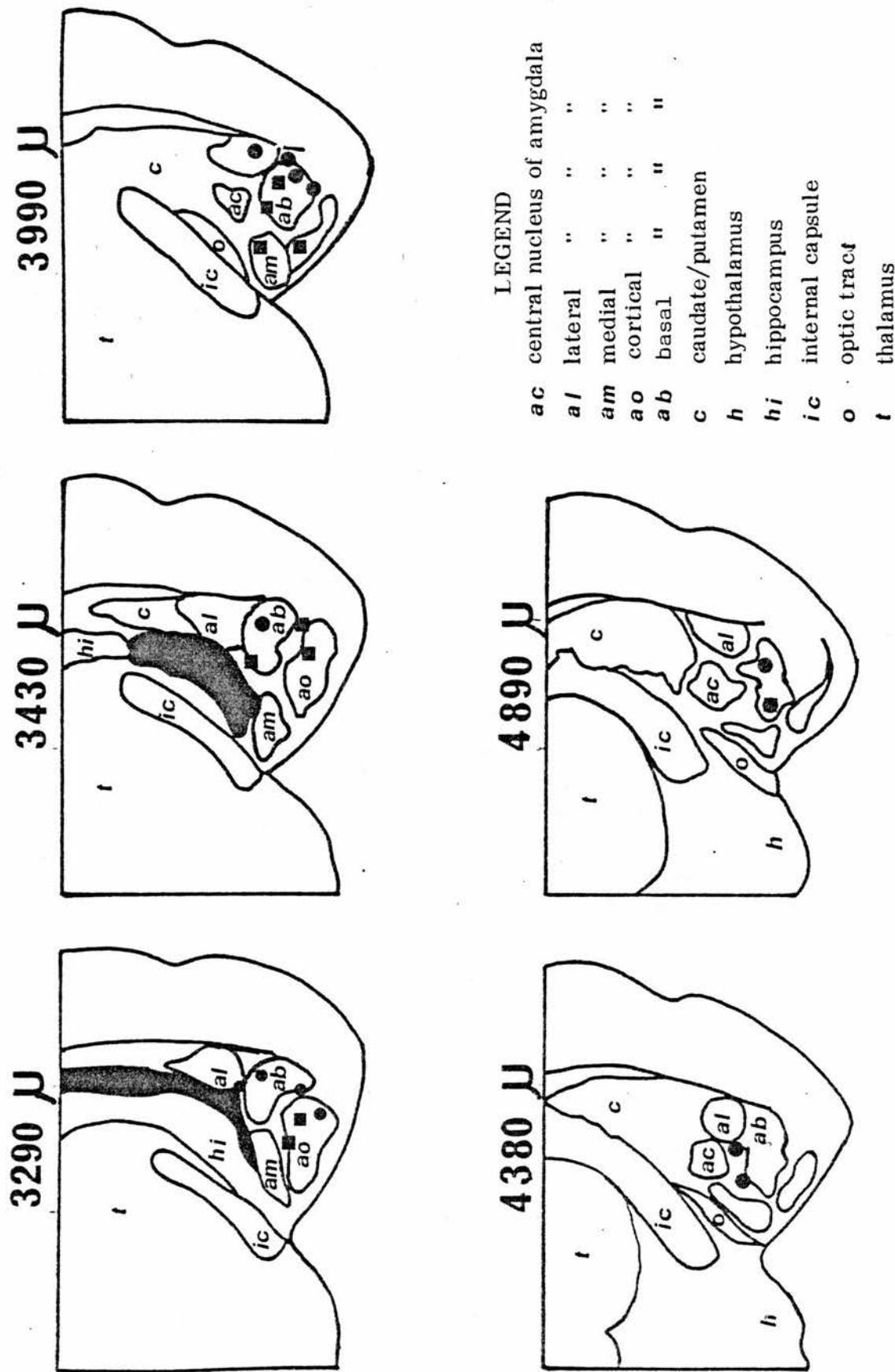
The distribution of electrodes is similar in rats which were kindled after 1 week and after 1 month.

THE RATE OF KINDLING AFTER AN ELECTRODE HAS BEEN
IMPLANTED IN THE AMYGDALA FOR A PERIOD OF 1 WEEK AND
1 MONTH, USING TWO TYPES OF ELECTRODE

	<u>Stainless steel</u> <u>electrodes</u>				<u>Platinum</u> <u>electrodes</u>			
	\bar{x}	\pm	SD	(N)	\bar{x}	\pm	SD	(N)
Electrodes implanted								
6 - 8 days before start of stimulation	16.4	\pm	5.4	., (9)	18.0	\pm	5.2	(10)
<hr/>								
Electrodes implanted								
28 - 33 days before start of stimulation	11.1	\pm	3.5	(12)	12.0	\pm	2.3	(5)
<hr/>								

With both types of metal electrode, animals kindled more rapidly 1 month after operation than 1 week after operation. ($P < .05$ for stainless steel, $P < .02$ for platinum using Wilcoxon Rank Sum Test). The differences between the groups using the two metals at 1 week and at 1 month were not significant.

THE PLACEMENTS OF STAINLESS STEEL ELECTRODES IN 21 RATS



All histologically verified placements are plotted in this diagram. Sections were drawn from Konig and Klippel (1963).

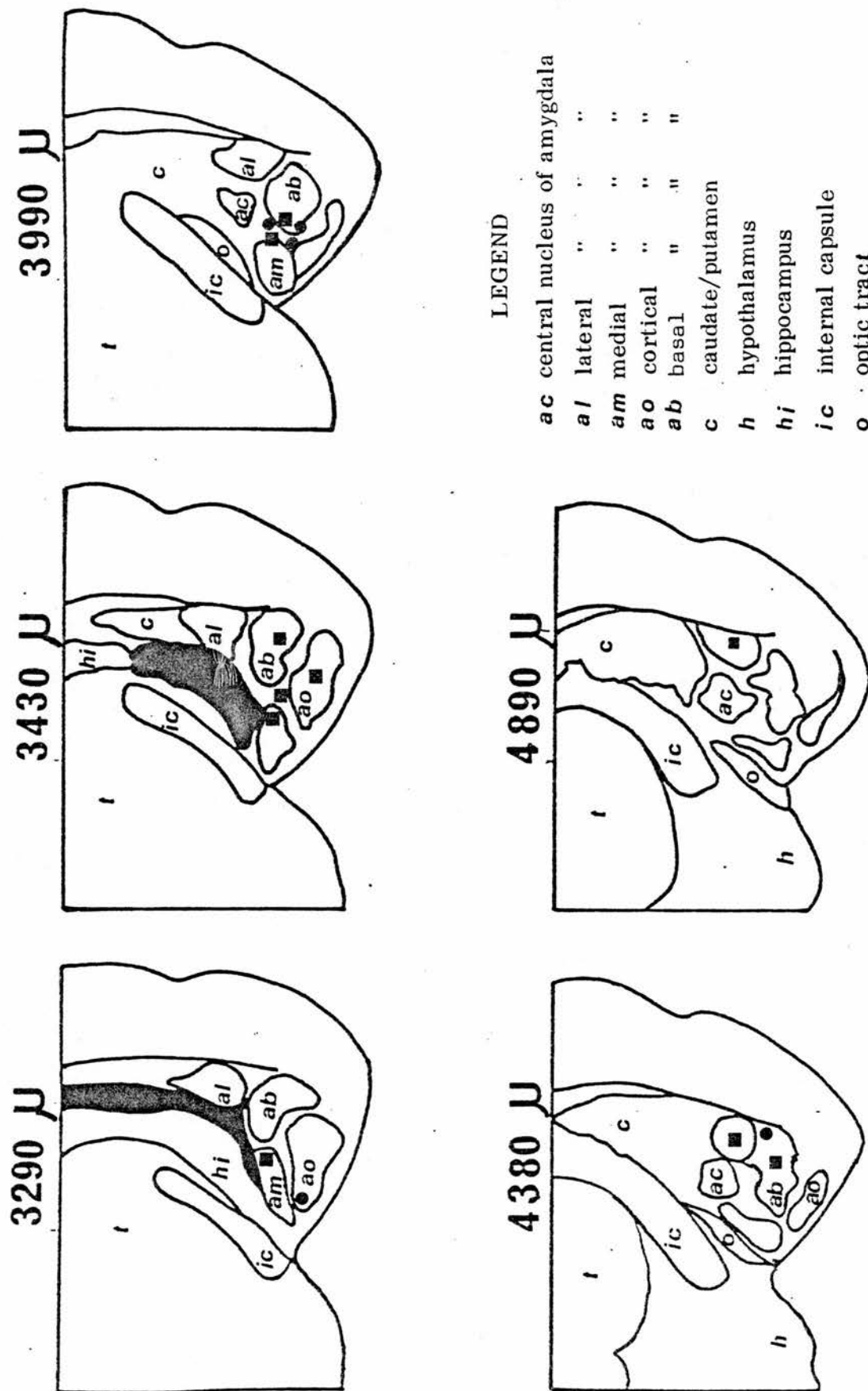
LEGEND

- ac* central nucleus of amygdala
- al* lateral " " "
- am* medial " " "
- ao* cortical " " "
- ab* basal " " "
- c* caudate/putamen
- h* hypothalamus
- hi* hippocampus
- ic* internal capsule
- o* optic tract
- t* thalamus

- Stimulation started after 1 week (9)
- Stimulation started after 1 month (12)

FIGURE 17

THE PLACEMENTS OF PLATINUM ELECTRODES IN 15 RATS



All histologically verified placements are plotted in this diagram. Sections were drawn from König and Klippel (1963).

3 - 3 DISCUSSION

THE EFFECT OF ELECTRODES THEMSELVES ON KINDLING

Although it has been well established that the chronic implantation of electrodes, constructed from a variety of materials, into different parts of the brain of a number of animal species may effect lasting biochemical (Robinson et al, 1975), histological (Robinson and Johnson, 1961) and behavioural (Hertz et al, 194, Boast et al, 1976) alterations, the effect of electrode implantation in kindling has been largely ignored.

It appears to have been tacitly assumed that electrode implantation of itself causes no effect on the rate of amygdaloid kindling and the error of this assumption is particularly relevant to a discussion of the so-called transfer effect (see Section 1 - 7). Curiously, Racine (1978), mentioning the possibility of an electrode effect, dismisses it, stating that it has been tested informally in many laboratories.

The enhanced rate of kindling we have observed with both stainless steel and platinum electrodes after one month of implantation could be sufficient to explain the phenomenon described as positive transfer. This hypothesis could be tested by implanting an electrode into the left amygdala and establishing the rate of primary kindling starting one week after operation. Then a second electrode would be placed in the right amygdala and kindling commenced one week later. If the rate was the same on both sides, it could be assumed that a transfer effect did not take place.

However, if the rate of kindling differed, it could still be argued that the traumatic damage caused by the electrode in the left amygdala had caused a "mirror focus" response on the right, and it is essential by the use of adequate controls to separate the relatively minor effects due to the trauma of electrode implantation from the major effects of electrical stimulation in kindling.

Electrical stimulation itself, at the energies used in kindling, does not appear to cause any tissue damage in the rat (Robinson et al, 1975) or monkey (Delgado, 1961 and 1977), but several theories can be proposed to explain the effect of metal electrode implantation.

(a) The Deposition of Iron and the Interruption of Brain Vasculature Following Electrode Implantation

Boast et al (1976) demonstrated that a specific behavioural effect, a defect in learning processes in mice, could be caused merely by the implantation of either stainless steel or platinum/iridium electrodes into the dentate region of the hippocampus. Furthermore, the memory impairment correlated with the appearance of Fe^{3+} around the tip of both types of electrode. Other behavioural alterations following brain manipulation have been correlated with iron accumulation even following the use of platinum electrodes (Dahl and Ursin, 1969).

Boast et al (1976) not only demonstrated by histochemical methods and by electron beam microprobe X-ray analyzer that Fe^{3+} is located in neural tissue directly adjacent to the track of the electrode implanted in the mouse hippocampus, but they also visualized the vascular tree of the hippocampus using india ink and demonstrated that vascular damage did occur and was closely related to the appearance of Fe^{3+} .

They concluded that iron deposition could be a consequence of blood trailing down the path of the electrode during insertion, but more importantly could be secondary to a fairly minor disruption of blood flow in the microvasculature of the implanted area. Vascular damage could result in ischaemia, oedema or biochemical change.

In view of the close correlation between regional blood flow and function in the brain, this explanation of the effect of electrode implantation on behaviour is most attractive and receives support from my own findings using deoxyglucose, which show an electrode effect on glucose uptake at the region of implantation and related structures.

(b) Tissue Response to the Metal

Fischer et al (1961) compared the histological reaction to wires of silver, copper and stainless steel with four different insulating materials implanted into brain tissue of cats. Stainless steel was tolerated extremely well and produced lesions limited to the extent of the mechanical trauma produced by their insertion.

The work was repeated and extended by Robinson and Johnson(1961), who found in cats that stainless steel rods caused the least tissue reaction

over a period of 6 months, while platinum caused a slightly larger lesion. After 15 days, the reaction to stainless steel consisted of some demyelination connective tissue encapsulation with scant glial cell infiltration. In the lesion produced by platinum rods there were hemosiderin deposits with a few erythrocytes free in the tissue.

Because platinum causes a more severe tissue response than stainless steel, my findings that the effect on kindling is the same with both types of metal electrodes, taken together with the evidence against any histological lesion in kindled rats (Goddard, 1969, Racine, 1976), would suggest that tissue damage is not significant as a cause of kindling.

The deposition of iron in the rat brain is capable of establishing an epileptic focus akin to the effect of cobalt, but much more lasting (Willmore et al, 1978). Following a subpial injection of 100 μ mol of ferrous or ferric chloride, rats developed bursts of epileptiform activity which persisted from 10 days to 12 weeks. Furthermore, Rosen and Frumin (1979) recorded focal spike activity, without behavioural seizures, within 48 hours of the injection of 10 μ l of a 13 mg μ solution of haemoglobin in saline, 1.5 mm. below the rat cortical surface. The spiking persisted for up to two months. It can be postulated that the enhancement of kindling by electrode insertion is due to a chronic focal epileptiform discharge due to the accumulation of iron or other component of haemoglobin around the electrode track.

(c) Biochemical Alterations in the Region of an Electrode Track in the Brain

Robinson et al (1975) implanted platinum electrodes into the rat hippocampus for periods of up to 60 days and examined for enzyme changes in the region of the electrode track. Most enzymes underwent changes which were accompanied by only very limited cell necrosis. The enzyme alterations reflected changes in both neuronal and glial activity.

A loss of nerve cell oxidative enzymes (lactic dehydrogenase, glucose-6-phosphate dehydrogenase) occurred within the first two days and persisted for 60 days and suggests a decreased rate of metabolism in neuronal tissue adjacent to the electrode.

At the same time, an increase in lysosomal activity was detected as an increase in acid phosphatase and glial cell activity was increased as shown

by enhanced activity in oxidative enzymes. In view of the findings of Boast et al (1976) mentioned above, it is noteworthy that alkaline phosphatase was elevated in the vascular endothelium, reflecting structural changes in blood vessels following injury.

These biochemical changes were not influenced by electrical stimulation and are further evidence that lasting changes in both neurones and glia may follow electrode implantation per se.

(d) It is likely that the presence of a conducting metal in the brain will have some influence on the activity of the cells in the vicinity of the metal. Surface charge effects may create potentials at the tip of a metal electrode, although the magnitude of this effect is likely to be very small. Adey (1975) has suggested that extremely small electric fields, causing tissue gradients far less than those required for postsynaptic activation, can interact with neuronal tissue and be detected through behavioural changes in animals.

Conclusion

The results presented here suggest that metal electrodes have a time-dependent effect on the amygdala, which enhances the rate of kindling. Furthermore the same effect is observed using teflon coated stainless steel and teflon coated platinum/iridium electrodes.

These results should be considered together with the results presented in Tables 15 and 16 from rats used to test the effect of atropine on kindling. In the latter experiment the observation of an electrode effect was serendipitous, and the effect was much more marked than in the subsequent experiments to confirm the finding. No histological verification of the electrode site was obtained from the first groups of rats, however, and the different rates of kindling may have been due to different electrode positions.

It is of interest that in my total experience of kindling the only rat which was fully kindled from the first stimulus had had an electrode in place for 25 days (Table 16).

In view of the normal biochemistry in the stimulated amygdala reported in this thesis and the normal histological appearance at the electrode site (Racine, 1978), the most likely explanation for the electrode effect is deposition of iron or interruption of vasculature.

THE 'TRANSFER EFFECT AS AN 'ELECTRODE' EFFECT

In the confusing literature on 'transfer' and 'negative transfer' in kindling there has been no systematic investigation of an electrode effect (Racine, 1972b, McIntyre and Goddard, 1973, McIntyre, 1976, Burnham, 1976, McCaughran et al, 1977, Gaito, 1976, Nobrega and Gaito, 1978, Messenheimer et al, 1979). Some features have emerged which support the hypothesis that positive transfer is artefactual and purely a local electrode effect.

1. McIntyre and Goddard (1975) noted that the amount of transfer was greater in rats which had long intervals between primary and secondary kindling. A two-week rest caused more 'transfer' (p. 538).
2. The 'transfer' effect was not interrupted by total commissurotomy (McIntyre, 1976). Furthermore, a local electrode effect could underlie the puzzling differences in latencies - the time lapse between the stimulus and the appearance of behavioural convulsions - observed in primary and secondary site kindling from the amygdala (termed the 'oscillation effect' by Gaito, 1976). The latency effect is not altered by complete commissurotomy and the simplest hypothesis, considered and rejected by Nobrega and Gaito (1978), that the effect is unilateral in nature and results from intrinsic differences between the two hemispheres, is given added weight if it is accepted that the mere presence of an electrode can cause a significant local effect on kindling.

McIntyre and Goddard (1973) used the 'transfer' effect as evidence that convulsions from both amygdalae share a final common pathway and that stimulation of one side forms a trace which is utilized after a small number of stimulations by the contralateral side. This seems unlikely, since split brain rats have unilateral seizures when either amygdala is stimulated.

The 'negative transfer' effect is based on firmer evidence and may be due to a reciprocal tonic inhibitory effect between homologous limbic sites. The enhancement of kindling in split brain animals may be due to the severance of these inhibitory connections.

CHAPTER 4

4 - 1 INTRODUCTION

USE OF LABELLED 2-DEOXYGLUCOSE TO MEASURE REGIONAL CHANGES IN METABOLISM IN KINDLING

A technique for identifying brain regions which become metabolically more active under certain behavioural conditions makes use of the regional changes in glucose consumption that occur during central nervous activity.

Sokoloff et al (1977) described the use of ^{14}C -deoxyglucose, a tracer for the exchanges of glucose between plasma and brain and its phosphorylation in brain tissue by hexokinase, as the basis of a method for the quantitative autoradiographic measurement of local cerebral glucose consumption. The method relies on the biochemical properties of deoxyglucose, which is transported across the blood-brain barrier by the glucose carrier system and competes with glucose intracellularly for hexokinase, which converts it to deoxyglucose-6-phosphate (DOG-6P). DOG-6P is not released directly from brain tissue to blood in measureable amounts and because it lacks a C2 hydroxyl group it is not isomerized by phosphohexoseisomerase and thus cannot participate in further glycolytic reactions (Horton et al, 1973). However, DOG-6P is a substrate for glucose-6-phosphatase and small amounts of the monophosphate are hydrolysed by this route (Sokoloff et al, 1977 and Hawkins and Miller, 1978). Sokoloff has maintained that glucose-6-phosphatase is present in only very small amounts in brain and that dephosphorylation probably occurs to considerably less than 5% of the glycolytic flux in the rat brain. Over the course of 45 minutes there would, therefore, be an insignificant loss of DOG-6P from the cell. This proposal, which forms a basic assumption for quantitative autoradiography, has been challenged by Hawkins and Miller, who claim that the rate of loss of DOG-6P from the cell has been underestimated and that glucose-6-phosphatase is the most likely candidate to cause the loss.

However, even if upheld, this criticism of the quantitative autoradiographic method does not apply to the use of deoxyglucose for non-quantitative studies.

If the assumption is made that deoxyglucose-6-phosphate is trapped within a cell for a period of at least 45 minutes and that the rate of its

accumulation in the cell is proportional to the rate of glucose consumption, then neural activity can be gauged. It is essential, however, that deoxyglucose should be used in tracer amounts, because deoxyglucose-6-phosphate does compete with glucose-6-phosphate for phosphohexose isomerase and in sufficient concentration inhibits glycolysis (Horton et al, 1973).

Application of the Deoxyglucose Technique

It has been amply confirmed in the brain that regional glucose utilization is finely regulated by neuronal activity (Sokoloff, 1977, Ingvar and Lassen, 1975).

Activity related alterations in the regional distribution of deoxyglucose were first shown when well defined sensory inputs were manipulated. Sciatic nerve stimulation in the rat caused an increased glucose consumption in the ipsilateral dorsal horn of the lumbar spine (Kennedy et al, 1975) and in the rat olfactory system stimulation with amyl acetate and other odourous chemicals produced a variety of patterns of increased deoxyglucose labelling (Sharp et al, 1975). Auditory deprivation in the rat by wax occlusion of the external auditory canals depressed the metabolic activity of all components of the auditory system (Sokoloff, 1977). Enucleation of an eye caused a decrement in glucose utilization in the visual system of the rat (Kennedy et al, 1976), while visual stimulation increased metabolism in regions of the visual system in the rat (Morjaria and Voaden, 1979), goldfish (Altenau and Agranoff, 1979) and the fruit fly, *drosophila* (Buchner et al, 1979).

However, when the deoxyglucose technique has been applied to the problem of identifying the sites of action of centrally acting drugs, results have frequently been much less straight forward.

Some drugs cause very widespread and seemingly non-specific changes in deoxyglucose distribution. These include systemically administered anaesthetic agents (Sokoloff, 1977), hydroxybutyrate (Wolfson et al, 1977), D-lysergic acid diethylamide (Shinohara et al, 1976) and morphine (Sakurada et al, 1976), or β endorphin, when injected into the region of the periaqueductal grey matter (Sakurada et al, 1978). Thus little information is obtained about the major sites of action of these agents. In the instance of PHENCYCLIDINE (Meibach et al, 1979) a specific distribution of change was found limited to limbic structures, and Savaki et al (1978) have shown specific

and opposite effects of α and β adrenoreceptor blockers on deoxyglucose distribution in the central auditory system, so the method may sometimes provide useful cues to drug actions.

Attempts to relate the functional changes visualized by deoxyglucose with biochemical and behavioural changes associated with specific neurotransmitter systems have been only partially successful.

The dopamine agonist apomorphine, when administered to rats, caused increased glucose utilization in two areas known to contain dopamine terminals and receptors, the substantia nigra and the caudate/putamen. Uptake was also increased in the globus pallidus and the sub-thalamic nucleus, for which there is little evidence for dopaminergic innervation. In all four regions the changes were blocked by pretreatment with haloperidol (Brown and Wolfson, 1978).

McCulloch et al (1979) in a further study using apomorphine also demonstrated increased deoxyglucose accumulation in caudate/putamen and substantia nigra, but additionally found that metabolism was increased in the frontal motor cortex - a region lacking both in dopamine uptake sites and in dopamine sensitive adenyl cyclase activity. This part of the cortex could be secondarily involved due to dopaminergic stimulation of the thalamus, so that secondary involvement of structures contributes to the non-specificity of deoxyglucose in pharmacological studies.

A second dopaminergic agonist, d-amphetamine, caused discrete and selective deoxyglucose uptake in known components of dopaminergic pathways, including all structures of the extrapyramidal motor pathway (Wechsler et al, 1979). The two dopamine agonists, D-amphetamine and apomorphine, gave very similar results. An outstanding feature in common in all three studies was the complete absence of increased deoxyglucose accumulation in any component of the mesolimbic dopaminergic system, even though nucleus accumbens was specifically examined. This is despite the biochemical evidence that both the striatal and mesolimbic dopaminergic system contain the same classes of dopamine receptors which respond in the same way to agonists and antagonists, and the evidence to suggest that the mesolimbic dopaminergic system is activated by the doses used of d-amphetamine and apomorphine (Iversen, 1977).

The results show only that the deoxyglucose technique relates to func-

tional changes, since although the mesolimbic dopaminergic system responds pharmacologically and biochemically in much the same way as the nigro-striatal system, it appears to be the striatal response which dominates and which becomes manifest in the rat's behaviour under those conditions of stimulus.

Further limitations of the technique when applied to the study of changes in activity of neurotransmitters were discussed by Schwartz (1978a), who in the course of examining the effects of a lateral hypothalamic lesion on regional glucose consumption observed a lowered deoxyglucose accumulation in the septal nuclei. However, the lesion he was making has been reported to increase the firing rate of certain neurones in the septal nuclei so that, while overall glucose metabolism in that region was reduced, certain cells within the region could have increased activity.

The regional glucose utilization is an average of all the energy requiring processes, including ion pumping, neurotransmitter synthesis, release and re-uptake and the synthesis of other molecules, so that changes in any one component may be masked by changes in others.

Stimulation of the locus coeruleus caused a decreased accumulation of deoxyglucose^{GP} in the cortex (Abraham et al, 1979) and a lesion of locus coeruleus was followed by a cortical increase in tracer (Schwartz, 1978b). However, neither author could measure either an increase or decrease in deoxyglucose subcortically, although it is well established that the procedures they used are followed by widespread changes in limbic noradrenergic function (Zigmond, 1979). Either the time scale of changes may be too long or the deoxyglucose technique as it stands is too insensitive to measure these neurotransmitter changes (Delanoy and Dunn, 1978).

Thus the distribution of deoxyglucose may not be trivially related to neurotransmitter activity, measuring as it does functional changes which represent an average of all the energy requiring processes which are taking place. It will require new techniques to increase the sensitivity of the method. For example, Basinger et al (1979) have improved resolution by using ³H-deoxyglucose and preparing serial 1 μ m tissue slices by plastic embedding for autoradiography. They have been the first to demonstrate ³H-2-deoxyglucose labelling in individually identified cells using the goldfish retina.

Deoxyglucose Used to Study Epileptic Spread

The spread of discharge from an epileptic focus is a process which is associated with an increase in the blood flow and metabolism in the tissues involved (Penfield et al, 1939, Schmidt et al, 1945, Plum et al, 1968) and recently a number of investigators have applied the autoradiographic ^{14}C deoxyglucose technique to study the metabolic changes in a variety of epilepsy models. Focal cortical epilepsy was first examined by Kennedy et al (1975) in monkeys, but a more thorough exploitation of the technique applied to penicillin-induced cortical epilepsy has been carried out by Collins in the rat (Collins et al, 1976, Collins, 1978a, 1978b). Metabolic changes within the cortex itself as well as in distant structures were analysed following a single injection of penicillin into the cortex, and also repeated after spaced small injections which induce a kindling type of response. Kindling from the amygdala in a small number of rats was studied using the deoxyglucose autoradiographic technique by Engel et al (1978) and the kindling effect of repeated systemic administration of lidocaine was used as a model by Ingvar and Ingvar (1979) and Post et al (1979). Myers and Shapiro (1979) studied changes during ictal phenomena induced by the anaesthetic agent enflurane.

During lidocaine kindled seizures Post et al (1979) found a reduced uptake of deoxyglucose in many regions, presumably due to the anaesthetic effect of the drug. Prolonged seizures were accompanied by increased uptake of glucose in the hippocampus, dentate gyrus, amygdala, septal nuclei and entorhinal cortex. However, the results are difficult to interpret on account of the overlap of two processes, the anaesthetic effect of the drug and the effect of seizures themselves.

In my own experiments I have examined the relative distribution of ^3H -deoxyglucose in selected regions of the rat brain following convulsions caused by amygdaloid kindling and by electroshock and compared these findings with operated and unoperated controls. My intention has been to compare and contrast the pattern of metabolic changes shown up by this technique with neurochemical, pharmacological, depth electrode and lesion experiments in amygdaloid kindling, on which a large literature has accumulated.

4 - 2 METHODS(³H) DEOXYGLUCOSE METHOD TO MEASURE REGIONAL GLUCOSE UTILIZATION DURING KINDLINGAnimals Used in These Experiments

Six sets of rats were used in these experiments, as follows:

Set 1

Eleven unoperated control rats.

Set 2

Nine sham-operated controls. These had an electrode stereotaxically placed in the left amygdala and were treated in all respects as kindled rats, excepting that they were not electrically stimulated. They served as a control for the effects of operation and handling. All rats retained their electrodes for the duration of the experiment.

Set 3

Six rats were fully kindled in the usual manner. Following the injection of deoxyglucose, these rats were not stimulated and hence had no convulsion. All the animals retained their electrodes to the time of death and they served as controls for all of the effects of kindling, including a history of at least 3 convulsions.

Set 4

Six rats with electrodes in the left amygdala were stimulated daily until they reached Stage 2 kindling with jaw movement, but without forelimb clonus (one rat did develop clonus during its final stimulation after deoxyglucose injection). These were used to study the effects of partial kindling. They were stimulated 2 minutes after DOG injection.

Set 5

Nine rats were fully kindled in the normal way and 2 minutes following deoxyglucose injection were stimulated to evoke a generalized convulsion. Rats which did not have a generalized convulsion following their final stimulation were excluded from the study. Stress was the most likely cause of a failure to convulse after an injection (for instance, see Pinel et al, 1973).

Set 6

Ten unoperated rats received electroconvulsive shock 2 minutes after deoxyglucose injection by the following procedure.

A portable clinical ECT generator which produced a fixed 150 v. sine wave stimulus with a current of 2.2 amps on open circuit was used. The resistance of the circuit formed when a current was passed through a rat's head via crocodile clips attached to the ears was measured in two anaesthetized rats and found to be in the region of 5000 - 5500 ohms, giving a current in the region of 30 ma. It was then verified on an Avometer that, when the 150 v. stimulus was applied across the pinnae for 1 second (with electrical contact enhanced by saline), the current did not exceed 50 ma.

All animals in this set received a stimulus of 150 v. for 1 second, receiving a maximum of 50 m. coulombs applied between the ears.

Procedure for the Intravenous Injection of Deoxyglucose

Rats were gently restrained in a purpose-built perspex holder, with their tail projecting free.

About 1 ml. of 2% lidocaine was injected subcutaneously around the root of the tail, which was thus completely anaesthetized by ring block. The tail could then be manipulated and incised without causing animal discomfort. A half-inch longitudinal skin incision was made with a scalpel near the base of the tail immediately to one side of the midline. Soft tissue dissection with blunt forceps and a seeker exposed the dorsal and the lateral tail vein, which could be dilated by reflex heating when the tip of the tail was immersed in warm water. Using a 25 gauge needle and a small syringe, 300 μ l of a solution of deoxyglucose in saline was injected by venepuncture into one of the exposed veins, usually the lateral one. By this procedure stress was minimized and the reliability of the intravenous administration of deoxyglucose could be directly observed.

In a small number of animals the intravenous injection was not successful and the drug was given by intraperitoneal injection. Inspection of the results subsequently suggested that the relative distribution of tritium in regions of the brain had not been affected by the route of injection and possibly the intraperitoneal route, despite the lower blood and brain levels of deoxyglucose achieved, would be a future method of choice.

Dose of Deoxyglucose

The following batches of commercially available tritiated deoxyglucose were used in these experiments:

<u>Preparation</u>	<u>Batch No.</u>	<u>Specific Activity</u>
2-deoxy-D-(1 - ^3H) glucose (The Radiochemical Centre, Amersham, TRK 383)	19	17.5 Ci/m mol
	20	15.4 Ci/m mol
	21	18.8 Ci/m mol
2-deoxy-D-(1, 2, ^3H) ^{GLUCOSE} Net 549 (New England Nuclear)		40 Ci/m mol

All animals were administered a dose of 200 $\mu\text{Ci/kg}$ made up to a total volume of 300 μl with 0.9% saline. The concentration of deoxyglucose was, therefore, not identical in all animals and the proportion of ethanol in each injection also varied slightly. However, these differences were considered not significant.

The Estimation of Regional Deoxyglucose-6-Phosphate Accumulation

Two minutes after the tail vein injection of deoxyglucose, animals were placed individually in the perspex stimulating box and, apart from the unoperated controls, attached to stimulating leads. Sets 4, 5 and 6 received a stimulus and the character and duration of the ictal events were recorded and after-discharge duration of the kindled and part-kindled rats was measured. On removal from the stimulating box, rats were individually housed in cages in a warm, quiet room with subdued lighting. In almost all cases the animals rested and performed minimal activity during this period.

45 minutes after injection of isotope the rats were killed by a blow on the thorax, decapitated and the whole brain was removed, quickly rinsed in a large volume of ice-cold saline, placed on a glass slide and frozen to -74°C on solid CO_2 (Drikold).

The frozen brain was cut into approximately 1 mm. slices using a hand-held razor blade and the slices were laid on glass microscope slides and left frozen on drikold until dissection. The dissection of each slice was performed with the aid of a dissecting microscope, while slices were kept at $0 - 4^{\circ}\text{C}$ on a Lager cooler. The relevant brain areas were identified using the rat brain atlas (Konig and Klippel) and dissected tissue was placed in Ependorf tubes. Although the whole procedure for each brain took about $1\frac{1}{2}$ hours, no individual brain slice was thawed for more than 10 minutes to reduce the

diffusion of deoxyglucose in the tissue sample. The sample was homogenized in ependorf tubes in 100 μ l distilled water to which was added 1 μ l octanol to reduce frothing (Griffin and George homogenizer at 1000 - 2000rpm for 60 seconds). A further 900 μ l distilled water was added to give a final volume of 1 ml., of which 800 μ l was removed to a scintillation vial, to which 10 ml. of NE 260 micellar scintillator was added. The activity of tritium in each sample was measured by liquid scintillation, counting for 10 minutes (Nuclear Chicago Mark II) at an efficiency of 37%. The remaining 200 μ l of homogenate was used for the estimation of protein by the method of Lowry et al.

Details of the Dissection

Numbers in the following paragraphs refer to the coordinates given in the stereotaxic atlas of the rat brain (Konig and Klippel, 1963).

Cortical terminology is that used in Craigie's Neuroanatomy of the Rat (1963) (Figure 1).

Olfactory Bulbs

After the brain was removed from the skull, the olfactory bulbs were retained in their bony cavity. The bulbs were scooped out. The olfactory tract and olfactory nerve were not used.

The tissue was variable in amount and always to some extent contaminated with blood vessels.

Fronto-parietal Cortex

From the level of the anterior extent of caudate/putamen (approx. A 9650 μ) to the level of the chiasma (approx. A 5650 μ).

After separation of the meninges, a rim of grey matter was removed from the dorsal and lateral surfaces and the inferolateral areas of pyriform cortex and the insular were excluded. The frontal pole was also excluded. The sample consisted mainly of parietal cortex with some frontal cortex and also dorsal sections of the anterior cingulate cortex. The motor cortex was included.

Occipital Cortex (approx. 3000 μ to 1600 μ)

From approx. 3000 μ to 1600 μ a rim of cortex was removed from the dorsal aspect of the cortex. The meninges were stripped off.

The section was almost entirely occipital cortex (visual cortex), but

may have included parts of posterior cingulate cortex medially. Temporal, pyriform and entorhinal cortex were excluded, since the dissection did not extend to the occipital pole posteriorly nor to the paleocortex inferolaterally.

Septal Nuclei

The structures enclosed by the lateral ventricles, corpus callosum and anterior commissure between coordinates 9000 μ to 6800 μ .

The tissue consisted mainly of medial, lateral and triangular septal nuclei, but also included stria terminalis, the septo hypothalamic tract, diagonal band of Broca, cortico habenular tract and parts of the fornix.

Regions ventral to the level of the anterior commissure were not included (olfactory tubercle and preoptic nuclei).

Amygdala

All amygdala nuclei were dissected between coordinates 5800 μ and 2800 μ using the optic tract and external capsule as easily identifiable landmarks.

Hypothalamus

All hypothalamic nuclei were dissected between coordinates 4800 μ and 3750 μ .

Thalamus

The extent of the thalamus could be fairly easily visualized in fresh tissue and the dissection was between 5800 μ and 2800 μ .

Hippocampus

This structure was readily removed between the coordinates 5000 μ and 1600 μ . The dissection included alveus fimbria and dentate gyrus.

Caudate/Putamen/Globus Pallidus

The corpus striatum anterior to approximately 6000 μ was taken.

The dissection included the globus pallidus.

Superior Colliculus

Identified between coordinates A 2200 and P 100.

Inferior Colliculus

Between coordinates A 620 μ and P 480.

Substantia Nigra

Visible between coordinates A 2790 μ and 1500 μ .

Brain Stem Reticular Formation

This structure was identified by its relations with the aqueduct of Sylvius and the central grey matter medially and the medial geniculate body and inferior colliculus laterally. It was dissected between the coordinates A 2000 μ and A 1000 μ .

Cerebellar Hemispheres

Samples of all the lobes of both cerebellar hemispheres were removed and care was taken to separate the overlying meninges.

Cerebellar Nuclei

The grey matter of the cerebellum embedded near the roof of the fourth ventricle was removed and material from right and left sides was combined.

The tissue included the dentate, emboliform, globose and fastigial nuclei since individual nuclei could not be adequately identified or separated.

Calculation of Results

The quantity of tritium (which, as Sokoloff has shown, is mainly incorporated into intracellular deoxyglucose - 6 - phosphate by this stage) in each brain region was expressed as c.p.m. per mg. protein.

The mean value obtained from the right and left visual cortex was chosen as a reference level for each animal and the specific activity for all other brain areas were expressed as a percentage of that value to give a relative measure of glucose uptake.

When the values obtained for right and left visual cortex in one animal differed by more than 12% the results from the whole animal were discarded.

4 - 3 RESULTS

1. Comparison of I.V. and I.P. Injection

Figure 18 compares the radioactivity present in blood following injection of tritiated deoxyglucose by the intravenous or intraperitoneal route. I.V. injection peaks at 2 - 4 minutes and tails off to 30% of the peak by 45 minutes. I.P. injection, as expected, gives a less rapid rise and a lower peak value. Since the amount of labelled deoxyglucose entering neurones will be related to the area under the curve in Figure 18, I.V. injection will lead to a distribution of DOG which is weighted towards neuronal events occurring in the first 10 - 15 minutes following injection. I.P. administration, on the other hand, will cause the deoxyglucose distribution to reflect a mean of neural activity throughout 45 minutes with much less emphasis on events in the first 10 minutes.

2. Behavioural Responses Observed after Part-kindling, Kindling and ECS

Partially kindled rats (Set 4)

All rats developed repetitive ictal jaw and facial movements when stimulated following deoxyglucose injection, and after-discharges were recorded in every instance. One rat developed forelimb clonus for the first time during this trial, but it was felt that all animals were at almost the same stage of kindling (Stage II and almost Stage III).

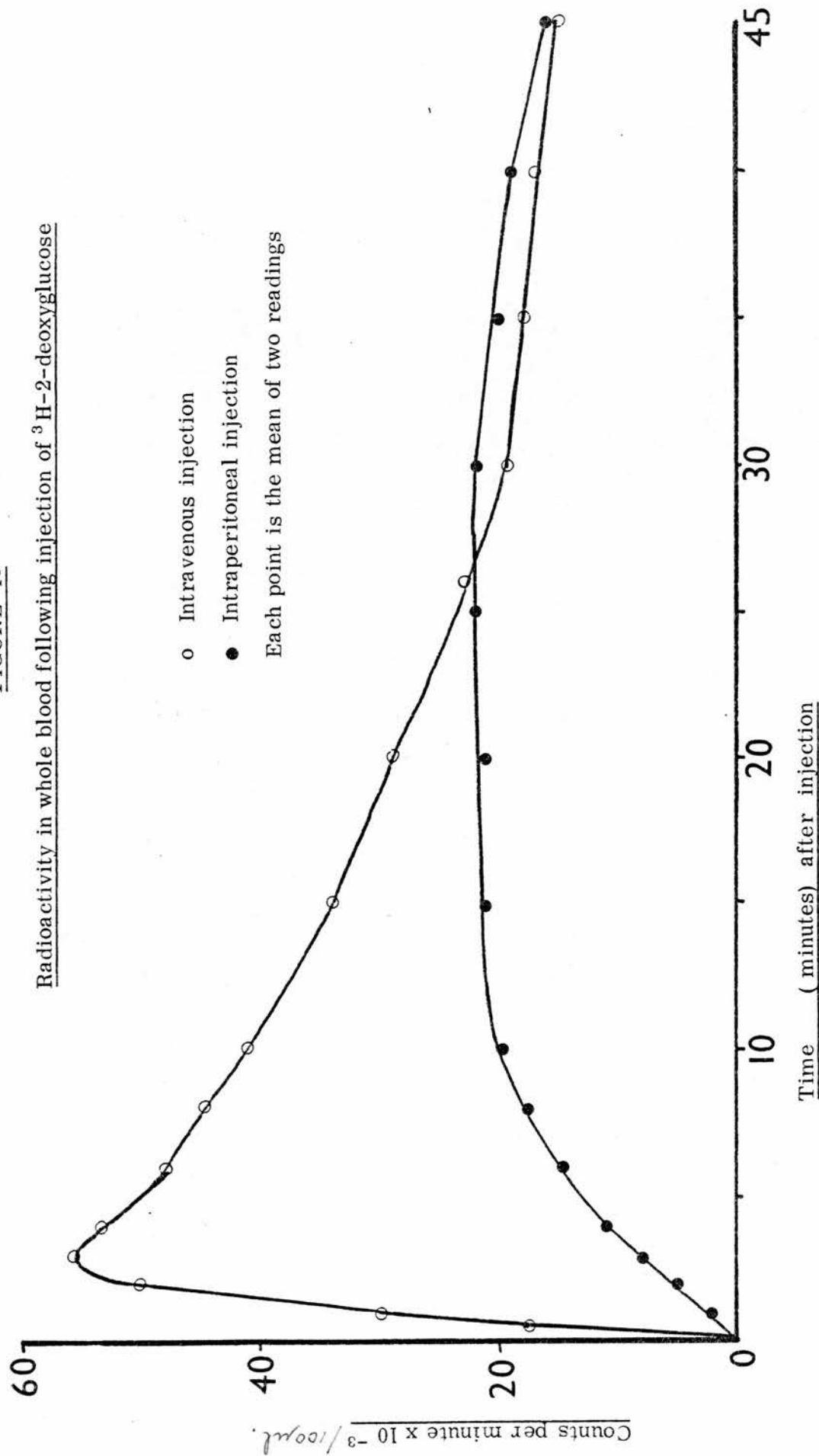
After-discharge durations during the final stimulus trial in the six animals ranged from 24 to 84 seconds (Mean 46 seconds).

Kindled rats (Set 5)

All rats responded to a stimulus with a full Stage V kindled seizure accompanied by an after-discharge duration of 25 - 120 seconds. Presumably because of stress, the seizure threshold was sometimes elevated and a higher than usual current was applied. This was never greater than 800 μ a.

FIGURE 18

Radioactivity in whole blood following injection of ^3H -2-deoxyglucose



The Response to Electroconvulsive Shock

All rats developed a generalized tonic clonic seizure which persisted for 20 - 60 seconds in most animals; in 1 rat clonus continued for 90 seconds.

The limb clonus was accompanied by a prominent lordosis and stiffness and arching of the tail; eyes were shut and there were frequently jaw movements and hypersalivation. In several rats the hyperactivity affected the right and left side asymmetrically for periods during the convulsion.

The tonic clonic phase was followed by a period of depression during which the animals lay quietly on their right or left side for 1 - 5 minutes, before gradually recovering normal exploratory behaviour patterns. Recovery was complete in about 10 minutes.

The convulsions induced by electroshock appeared totally different in several respects from kindled convulsions.

1. Electroshock convulsions enter immediately into the tonic clonic phase, whereas during the first few seconds of kindled convulsions there is generally a progression of ictal features which closely recapitulate the stages of the development of kindling.
2. In electroshock the clonic phase involves hind limbs as well as forelimbs. In kindling clonus is mainly in the forelimbs. The typical appearance of rearing and falling seen in kindling was not observed after electroshock, whereas the marked lordosis and extreme and sometimes prolonged tail hyperactivity are not features of kindling.
3. The recovery from kindled convulsions is frequently extremely rapid and rats resume exploration within seconds of the end of a generalized seizure. Following electroshock, animals were drowsy for several minutes. However, this difference may be accounted for by the higher current used in electroshock.

3. Statistical Analysis of the Deoxyglucose Data

The mean, standard deviation and number of samples of each brain area is given in Table 24. The individual values of relative glucose uptake in each brain region, from which Table 24 is derived, were used in a one-way analysis of variance (Anova) to test for significant differences amongst the means.

The Anova table constructed for each brain region is given in Table 25 (Reference: Snedecor and Cochran, 1967).

There was no significant difference between groups in the right and left fronto-parietal cortex and the right and left olfactory bulb. Therefore, no further analysis was required on the data from these regions.

Analysis of variance showed that significant differences did exist between treatment groups in all other brain regions and further analysis was to identify which of the groups differed significantly from the rest.

Inspection of all the data revealed that right and left sides were similar. This was confirmed by applying the paired t-test, which showed that in no treatment group was there a significant difference between the right and left sides of any brain region.

Therefore, for subsequent analyses the best estimate of the standard error of a mean for a particular region was obtained by combining the residual (error) mean squares (given in the Anova table) from right and left sides. Similarly the means and degrees of freedom of right and left sides were combined.

The student-Newman-Keul procedure (Sokal and Rohlf, 1969) was adopted for making multiple comparisons, a posteriori, among means based on unequal sample size. The test employs the studentized range and is a sequential method. The largest difference found among a given set of means (their range) is used as the statistic for measuring the significance of the difference.

$Q_{(af)}$ obtained from tables is the critical value, at a given level of significance, of the studentized range applied to (a) means with (f) degrees of freedom.

$$\text{And } Q_{(af)} = \frac{\bar{x}_{\max} - \bar{x}_{\min}}{S\bar{x}}$$

where \bar{x}_{\max} is the largest mean in the group of (a) means

\bar{x}_{\min} is the smallest mean in the group of (a) means

$S\bar{x}$ is an estimate of the pooled standard error of the whole group of means.

For a difference between means to be significant at a given level

$$\bar{x}_{\max} - \bar{x}_{\min} \geq Q_{(af)} S\bar{x}$$

In other words, the least significant range (L.S.R.) is given by:

$$\text{LSR} = Q_{(af)} S\bar{x} \quad (\text{Equation 1})$$

The pooled standard error of a group of means ($S\bar{x}$) is estimated using the residual (error) mean squares given in the Anova table.

$$S\bar{x} = \sqrt{\frac{\text{Residual mean squares}}{n}} \quad (\text{Equation 2})$$

However, this applies only when sample sizes are all the same and equal to n .

Since the means in Table 24 are based on samples of unequal size, equation 2 must be modified by the replacement of n by:

$$\frac{2 n_1 n_2}{n_1 + n_2}$$

which gives an average of the two sample sizes in a given comparison.

$$\text{Thus: } S\bar{x} = \sqrt{\frac{\text{Residual mean squares}}{2 n_1 n_2}} \sqrt{\frac{N_1 + N_2}{2 n_1 n_2}} \quad (\text{Equation 3})$$

Substituting equation 3 in equation 1 we have:

$$L.S.R. = Q_{(af)} \sqrt{\frac{\text{Residual mean squares}}{2N_1 N_2}} \sqrt{\frac{N_1 + N_2}{2N_1 N_2}}$$

Thus for a difference between any two means in Table 24 to be significant it must be equal to or greater than the least significant range, tested in a step-wise manner.

The means are tabulated in order of size and first the largest range (smallest and largest mean) is tested by comparing it with L.S.R. for (a) means. If this difference is not significant none of the differences enclosed by these means can be significant. If the maximum range is significant the next range is tested and compared with L.S.R. for (a - 1) means, and the appropriate degrees of freedom and so on. $S\bar{x}$ must be recalculated for each subgroup being compared, since n_1 and n_2 are variable. In each case Q for the appropriate number of means and degrees of freedom was obtained at the 5% level of significance. Higher levels of probability were not tested and the calculations were completed with the aid only of a pocket calculator.

As previously noted, right and left sides were combined to give an improved estimate of residual mean squares and means for the Student-Newman-Keul's test.

Diagrammatic representation of the results of the analysis is given in Figure 19, constructed at the 5% level of significance. In this figure the means are arranged in order of magnitude and the lines connect non-significant sets of means. Any pair of means enclosed by the range of a single line are not significantly different. Thus in the amygdala, set 1 (unoperated) does not differ from set 2 (sham-operated) or set 3 (kindled but not convulsed), but is significantly lower than set 4 (partially kindled), set 5 (kindled and convulsed) and set 6 (ECS). Also, the table shows that in the amygdala set 6 is significantly higher than all other sets, whereas set 5 is only significantly higher than set 1, 2 and 3.

Since sample sizes are unequal and the value of the least significant range reflects sample size (Equation 3), means in non-significant sets are not necessarily adjacent. This is indicated by arrows in the rows representing the superior and the inferior colliculus. In the superior colliculus sets 1, 2, 3 and 6 are not significantly different from each other, but these sets all differ significantly from sets 4 and 5.

TABLE 24

RELATIVE GLUCOSE UTILISATION IN VARIOUS
BRAIN REGIONS EXPRESSED AS A
PERCENTAGE OF THE VALUE OBTAINED IN EACH
ANIMAL FOR THE OCCIPITAL CORTEX
(Figures in each column are Mean S.D. N)

	Unoperated		Sham Operated		Kindled no convulsion		Partially Kindled		Kindled Convulsed		E.C.S.							
AMYGDALA R	69.1	4.9	11	70.9	5.6	9	73.8	6.0	6	80.2	6.0	6	82.2	5.4	9	93.3	7.7	10
	70.3	6.0	10	72.4	4.9	9	75.3	6.0	6	80.7	6.8	6	83.4	5.5	9	94.0	9.6	10
HIPPOCAMPUS R	70.0	3.2	11	74.7	4.1	9	78.8	5.6	6	84.	13.2	6	91.6	11.7	9	102.7	9.3	10
	71.3	5.8	10	75.1	3.7	9	81.0	7.6	6	83.	10.4	6	92.6	7.5	9	101.6	8.3	10
PARIETAL CORTEX R	100.1	5.1	11	102.7	8.9	9	101.8	6.0	6	96.2	7.4	6	98.6	3.7	9	101.5	4.0	8
	96.3	6.4	11	100.2	13.0	9	92.5	9.1	6	93.8	4.5	6	103.2	9.9	9	100.6	4.3	10
THALAMUS R	85.5	8.0	11	91.1	5.1	9	96.0	9.6	5	-	-	-	93.	8.4	8	95.	7.6	10
	81.5	6.4	11	89.0	5.3	9	94.8	7.4	6	-	-	-	94.9	7.2	9	94.5	9.0	10
HYPOTHALAMUS R	66.0	6.9	11	72.8	5.3	9	76.5	3.9	6	79.	3.2	6	82.3	6.1	9	86.	8.5	10
	66.6	7.6	11	72.3	5.2	9	76.3	2.9	6	79.2	8.4	6	81.7	6.1	9	86.4	9.7	10
STRIATUM R	83.1	6.9	11	85.9	6.2	9	94.6	7.5	5	-	-	-	94.2	6.1	9	95.6	9.8	10
	84.7	5.0	11	88.3	7.7	9	89.8	9.0	6	-	-	-	91.8	5.2	9	98.9	7.9	10
SEPTAL NUCLEI R	65.4	7.0	11	72.4	5.9	7	76.5	5.1	6	-	-	-	85.1	5.7	7	90.0	7.4	10
	66.2	6.0	11	74.4	8.2	7	76.3	4.6	6	-	-	-	90.9	9.8	7	91.4	7.0	10

TABLE 24 (continued)

RELATIVE GLUCOSE UTILISATION IN VARIOUS
BRAIN REGIONS EXPRESSED AS A
PERCENTAGE OF THE VALUE OBTAINED IN EACH
ANIMAL FOR THE OCCIPITAL CORTEX
(Figures in each column are Mean S.D. n)

	Unoperated	Sham Operated	Kindled no convulsion	Partially Kindled	Kindled Convulsed	E.C.S.
CEREBELLAR NUCLEI	76.1 8.5 8	85.1 7.2 9	87.6 3.3 5	-	88.9 9.3 8	96.2 9.6 9
CEREBELLAR R	68.1 5.2 11	73.4 8.6 9	74.4 6.4 5	-	78.4 7.8 9	81.5 11. 10
HEMISPHERES L	69.2 8.8 10	72.3 7.3 7	77.8 8.8 5	-	80.6 7.7 9	84. 16.1 10
RETICULAR R	77. 6.8 11	78.7 8.3 7	90. 3.4 6	-	87.1 10.7 9	94.9 8.8 9
FORMATION L	80.9 9.4 11	82. 7.6 7	87.2 4.4 5	-	88. 8.0 9	93.1 10.7 9
OLFACTORY R	74.5 9.0 10	75.3 10.6 9	84. 9.7 5	-	82.9 9.0 9	89.4 13.9 5
EULB L	71.6 8.1 11	77.1 10.2 9	75.2 13.1 5	-	80.8 10.6 9	82.7 28.1 6
INFERIOR R	98.2 6.7 11	99.6 16.9 8	101.3 10.1 6	-	114.6 15.1 9	109.6 10.7 10
COLLICULUS L	95.9 9.3 10	103.6 11.2 9	98.2 14.4 6	-	115. 13.7 9	108.1 9.2 10
SUPERIOR R	87.7 9.4 10	93.3 8.6 9	94.2 6.9 6	108.8 5.0 6	108. 7.3 9	97.6 6.4 10
COLLICULUS L	91.6 11.2 11	91. 5.9 8	90.8 7.4 6	100.8 8.9 6	105. 7.1 9	98.5 6.2 10
SUBSTANTIA NIGRA R	66.3 5.6 8	64.1 11.3 7	76.5 6.0 6	78.3 2.9 4	88. 19.6 9	86.1 18.6 9
SUBSTANTIA NIGRA L	65.3 5.6 8	65.7 14.0 6	72.2 4.6 6	80.3 3.6 4	93.3 13.9 8	91.8 16.5 9

TABLE 25

ANALYSIS OF VARIANCE (ONE WAY)
USING THE SAME DATA FROM
WHICH TABLE 24 WAS DERIVED

(When treatment degrees of freedom is given as 5, the 6 groups are: 1) Unoperated, 2) Sham-operated, 3) Kindled (but not convulsed) 4) Partially kindled, 5) Kindled and convulsed, 6) ECS. When df = 4 the partially kindled group was not assessed).

Region		Sum of Squares	Degrees of Freedom	Mean Square	F	Significance
RIGHT AMYGDALA	Treatments	3956.2	5	791.24	21.91	p < .01
	Error	1625.12	45	36.11		
	Total	5581.33	50			
LEFT AMYGDALA	Treatments	3648.31	5	729.66	16.04	p < .01
	Error	2001.21	44	45.48		
	Total	5649.52	49			

TABLE 25 (continued)
ANALYSIS OF VARIANCE (ONE WAY)
USING THE SAME DATA FROM
WHICH TABLE 24 WAS DERIVED

Region		Sum of Squares	Degrees of Freedom	Mean Square	F	Significance
RIGHT HIPPOCAMPUS	Treatments	7107.00	5	1421.4	20.43	p << .01
	Error	3131.16	45	69.58		
	Total	10238.16	50			
LEFT HIPPOCAMPUS	Treatments	6130.71	5	1226.14	23.4	p << .01
	Error	2305.61	44	52.4		
	Total	8436.32	49			
RIGHT THALAMUS	Treatments	645.13	4	161.28	2.77	p < .05
	Error	2215.62	38	58.31		
	Total	2860.74	42			
LEFT THALAMUS	Treatments	1365.05	4	341.26	6.71	p << .01
	Error	2032.95	40	50.82		
	Total	3398.0	44			

TABLE 25 (continued)

ANALYSIS OF VARIANCE (ONE WAY)
USING THE SAME DATA FROM
WHICH TABLE 24 WAS DERIVED

Region		Sum of Squares	Degrees of Freedom	Mean Square	F	Significance
RIGHT HYPOTHALAMUS	Treatments	2580.12	5	516.02	13.07	p << .01
	Error	1777.06	45	39.49		
	Total	4357.18	50			
LEFT HYPOTHALAMUS	Treatments	2505.88	5	501.18	9.7	p << .01
	Error	2325.29	45	51.67		
	Total	4831.8	50			
R. PARIETAL CORTEX	Treatments	206.26	5	41.25	1.13	n.s.
	Error	1566.8	43	36.44		
	Total	1773.06	48			
L. PARIETAL CORTEX	Treatments	670.95	5	134.19	1.87	n.s.
	Error	3230.03	45	71.78		
	Total	3900.98	50			

TABLE 25 (continued)

ANALYSIS OF VARIANCE (ONE WAY)
 USING THE SAME DATA FROM
 WHICH TABLE 24 WAS DERIVED

Region		Sum of Squares	Degrees of Freedom	Mean Square	F	Significance
RIGHT STRIATUM	Treatments	1256.7	4	314.2	5.66	p < .01
	Error	216.3	39	55.46		
	Total	3419.6	43			
LEFT STRIATUM	Treatments	1130.2	4	282.54	5.92	p < .01
	Error	1909.5	40	47.74		
	Total	3039.64	44			
RIGHT CEREBELLAR HEMISPHERE	Treatments	1077.7	4	269.44	4.03	p < .01
	Error	2605.1	39	66.8		
	Total	3682.8	43			
LEFT CEREBELLAR HEMISPHERE	Treatments	1981.11	4	495.28	4.31	p < .01
	Error	4136.5	36	114.9		
	Total	6117.56	40			

TABLE 25 (continued)

ANALYSIS OF VARIANCE (ONE WAY)
USING THE SAME DATA FROM
WHICH TABLE 24 WAS DERIVED

Region		Sum of Squares	Degrees of Freedom	Mean Square	F	Significance
RIGHT RETICULAR FORMATION	Treatments	2049.9	4	512.48	7.69	p << .01
	Error	2465.2	37	66.63		
	Total	4515.14	41			
LEFT RETICULAR FORMATION	Treatments	895.01	4	223.75	2.96	p < .05
	Error	2724.6	36	75.68		
	Total	3619.61	40			
RIGHT OLFACTORY BULB	Treatments	1094.99	4	273.75	2.64	n.s.
	Error	3418.59	33	103.59		
	Total	4513.58	37			
LEFT OLFACTORY BULB	Treatments	664.69	4	166.17	.83	n.s.
	Error	7009.31	35	200.27		
	Total	7674.0	39			

TABLE 25 (continued)

ANALYSIS OF VARIANCE (ONE WAY)
USING THE SAME DATA FROM
WHICH TABLE 24 WAS DERIVED

Region		Sum of Squares	Degrees of Freedom	Mean Square	F	Significance
CEREBELLAR NUCLEI	Treatments	1773.37	4	443.34	6.54	p << .01
	Error	2303.39	34	67.75		
	Total	4076.8	38			
R. SEPTAL NUCLEI	Treatments	3777.33	4	944.33	22.45	p << .01
	Error	1514.62	36	42.07		
	Total	5291.95	40			
L. SEPTAL NUCLEI	Treatments	4510.16	4	1127.54	21.5	p << .01
	Error	1887.94	36	52.44		
	Total	6398.1	40			

TABLE 25 (continued)

ANALYSIS OF VARIANCE (ONE WAY)
 USING THE SAME DATA FROM
 WHICH TABLE 24 WAS DERIVED

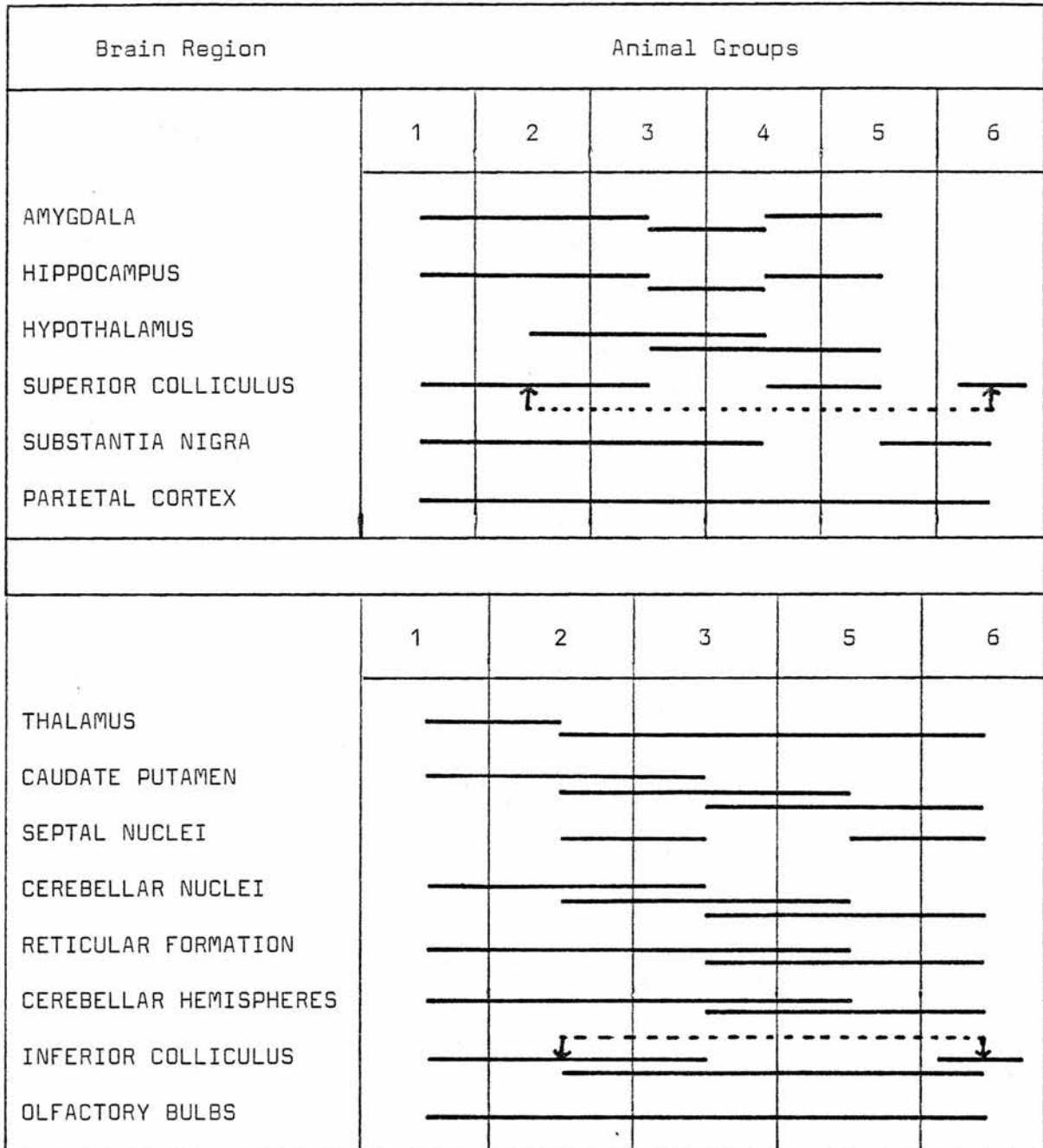
Region		Sum of Squares	Degrees of Freedom	Mean Square	F	Significance
R. INFERIOR COLLICULUS	Treatments	1855.1	4	463.77	3.11	p < .05
	Error	5821.47	39	149.27		
	Total	7676.55	43			
L. INFERIOR COLLICULUS	Treatments	2110.05	4	527.51	4.05	p < .01
	Error	5078.86	39	130.23		
	Total	7188.91	43			
R. SUPERIOR COLLICULUS	Treatments	2945.05	5	589.01	10.17	p << .01
	Error	2548.17	44	57.91		
	Total	5493.22	49			
L. SUPERIOR COLLICULUS	Treatments	1540.77	5	308.15	4.65	p < .01
	Error	2916.45	44	66.28		
	Total	4457.22	49			

TABLE 25 (continued)

ANALYSIS OF VARIANCE (ONE WAY)
 USING THE SAME DATA FROM
 WHICH TABLE 24 WAS DERIVED

Region		Sum of Squares	Degrees of Freedom	Mean Square	F	Significance
R. SUBSTANTIA NIGRA	Treatments	3946.13	5	789.23	4.16	p < .01
	Error	7018.38	37	189.69		
	Total	10964.51	42			
L. SUBSTANTIA NIGRA	Treatments	5959.31	5	1191.86	8.56	p < .01
	Error	4875.47	35	139.3		
	Total	10834.78	40			

FIGURE 19

GROUPS

- | | |
|--|--|
| 1. Unoperated controls | 4. Partially kindled |
| 2. Sham-operated controls | 5. Kindled. Convulsed after deoxyglucose injection |
| 3. Kindled rats not convulsed after deoxyglucose injection | 6. Electroconvulsive shock. |

A diagrammatic representation of the results of a multiple comparison test (Student-Newman-Keul) applied to the mean relative glucose utilization in the brain regions given in Table 24.

The mean glucose utilization in any two groups enclosed by the range of any one line are not significantly different. Groups not enclosed by the same line have mean glucose utilizations which differ significantly at the 5% level.

SUMMARY OF CHANGES IN GLUCOSE UTILIZATION DEPICTED IN
TABLE 24 AND FIGURE 19

1. Changes observed in glucose consumption were always bilaterally symmetrical. No significant right-left differences were found in any regions.

2. Regions showing no change

There was no significant difference in any of the groups tested in the relative glucose consumption in the fronto-parietal cortex or the olfactory bulbs. Relative to the occipital cortex these regions were uninvolved in kindling or in electroshock seizures.

3. The effect of electrode placement and the kindling procedure (sets 1, 2, 3)

In no area did the kindled (not convulsed) group differ from the sham-operated group. The kindled unconvulsed state is, therefore, not associated with higher energy consumption than sham-operated controls. However, in the hypothalamus and septal nuclei unoperated controls showed a significantly lower uptake than both sham-operated and kindled (not convulsed) animals.

Since these groups differ only in the placement of an electrode into the amygdala, the results could imply an electrode effect which is expressed by an *INCREASED* uptake in those regions. It is intriguing that whereas the electrode was unilateral the effect on deoxyglucose was observed bilaterally.

4. The effect of kindled convulsions

Set 5 differs significantly from both set 2 and 3 in the amygdala, hippocampus, superior colliculus, substantia nigra and the septal nuclei. Set 5 also differed from set 2 in the hypothalamus. There is no significant difference between kindled (convulsed) and sham-operated or kindled (not convulsed) sets in the thalamus, striatum, reticular formation, cerebellar hemispheres, cerebellar nuclei or inferior colliculus.

5. The effect of partial kindling

Only 6 brain regions were dissected bilaterally from rats which make up set 4. In all areas the mean relative glucose uptake was intermediate between kindled (not convulsed) and kindled (convulsed). It is noteworthy that even these results were always bilateral and the regions significantly

increased over sham-operated controls were amygdala, hippocampus and superior colliculus (but not substantia nigra).

6. The effect of electroconvulsive shock

Except for the inferior and superior colliculus, ECS always caused higher glucose utilization than kindling induced convulsions.

The best control for set 6 is set 1 and differences in glucose consumption attributable to ECS were found in all brain regions except fronto-parietal cortex, olfactory bulb, superior and inferior colliculus. The most marked changes were in the amygdala, hippocampus, and hypothalamus. It should be noted that the thalamus, striatum, cerebellar nuclei, cerebellar hemispheres and reticular formation, which were not affected by kindling induced convulsions, did demonstrate enhanced glucose consumption following ECS.

4. The Effect of Convulsions on Deoxyglucose-6-Phosphate Accumulation

Cerebral blood flow may increase by up to 9-fold during a prolonged seizure (Meldrum and Nilsson, 1976) and an increase in GLYCOLYSIS AND deoxyglucose accumulation in the brain is ^{ALSO} to be expected during seizures induced by kindling or electroshock. This was examined by comparing the values obtained for glucose utilization (expressed as cpm per mg. protein) in the visual cortex of the different sets of rats. A straight needle venepuncture has the advantage that stress is minimized and an operation to implant a cannula is avoided. A disadvantage is that an unknown amount of injecting solution may leak into surrounding tissue and all injections are, therefore, not the same. Table 26 gives mean counts per minute per unit of protein for the visual cortex of rats for which the intravenous injection was recorded at the time to be entirely satisfactory.

This rather approximate estimate of the effect of convulsions themselves on the accumulation of tritium in the visual cortex following DOG injection indicates that whole brain (or at least cortical) GLYCOLYSIS increases about 2.5-fold during a kindled convulsion and about 4-fold during an electroshock convulsion. The difference is not accounted for by a longer duration of seizure following electroshock. Indeed the reverse is true. After electroshock seizures lasted 20 - 60 seconds in all but 1 rat which convulsed for 90 seconds (Mean 40 secs.). The duration of the tonic clonic phase of kindled convulsions ranged from 25 to 120 seconds (Mean 78 secs.).

TABLE 26

CHANGES IN THE ABSOLUTE CONCENTRATION OF TRITIATED
DEOXYGLUCOSE DERIVATIVES DURING CONVULSIONS INDUCED
BY KINDLING AND ELECTROSHOCK

Accumulation of tritium in the visual cortex (mean of right and left) in $\mu\text{Ci./mg}$ protein, following tail vein injection of approximately 11 n moles tritiated deoxyglucose.

	<u>Unoperated and</u> <u>sham-operated</u> <u>rats</u>	<u>Kindled rats</u> <u>convulsed after</u> <u>D.O.G. injection</u>	<u>Rats given</u> <u>electroshock after</u> <u>D.O.G. injection</u>
\bar{x}	2.7	7.0	11.1
SD	0.9	1.4	1.3
N	16	8	4

Kindled convulsed = 2.6
 Unconvulsed controls

Electroshock = 4.1
 Unconvulsed controls

These results show that deoxyglucose - 6P accumulation in the visual cortex increases two and a half fold during a kindled convulsion and fourfold during an electroshock induced convulsion.

Metabolic Changes Consequent on Kindling1. The deoxyglucose technique

The theoretical basis for the utilization of deoxyglucose can be applied in several ways. Quantitative autoradiography requires the assumption of a steady state glucose utilization for 45 minutes and is, therefore, not appropriate to the quantification of changes during seizures. However, relative changes in regional glucose consumption can be measured in various animal modes of epilepsy.

I have described a method for measuring regional deoxyglucose consumption relative to occipital cortex. This region was chosen because studies with recording electrodes and lesion experiments have shown that the occipital cortex is not intimately involved with kindling from the amygdala and itself cannot be readily kindled (see Section 1 - 6).

A few general points can be made about the technique used in these experiments. Because we can measure only relative changes in glucose consumption and cannot fully exploit the advantage of deoxyglucose, ^{14}C glucose (Hawkins et al, 1976) could probably have been used just as effectively over a 10 minute as opposed to 45 minute period (Hawkins and Miller, 1978). However, using tritiated deoxyglucose this method is cheap and a single injection is unstressful, avoiding long periods of immobilization or anaesthesia. The dissection procedure, which is rapid and simple, appears to be a sensitive and reliable method for detecting small changes in glucose consumption.

To judge the reliability of the method, the results we have obtained for relative glucose uptake in various brain regions of unoperated control rats in Table 24 were compared with figures published by Sokoloff et al (1977) relating to unrestrained rats. The absolute levels of glucose consumption obtained by the quantitative autoradiographic method were converted to figures relative to the visual cortex (Table 27). The correlation between the two groups of figures was reasonably good ($r = 0.85$). The differences in certain areas could be explained by differences in dissection. For example, in dissection we included globus pallidus with caudate/

TABLE 27

LOCAL CEREBRAL GLUCOSE UTILIZATION DETERMINED
AT 45 MINUTES FOLLOWING A PULSE OF
DEOXYGLUCOSE IN THE NORMAL CONSCIOUS RAT

All results expressed as percent of visual cortex \pm SD

Brain Region	Quantitative Autoradiography (Sokoloff et al 1977) N = 10	Dissection of Brain Tissue (Mean of R-L Slides) own results N = 11
VISUAL CORTEX	100%	100%
AMYGDALA	49 \pm 6	70 \pm 5
HIPPOCAMPUS	74 \pm 9	71 \pm 4
PARIETAL CORTEX	104 \pm 16	98 \pm 5
THALAMUS	105 \pm 15	83 \pm 7
HYPOTHALAMUS	51 \pm 6	66 \pm 7
CAUDATE/PUTAMEN)	102 \pm 12)	84 \pm 6
GLOBUS PALLIDUS)	54.2 \pm 6.3)	
SEPTAL NUCLEI	60 \pm 9	66 \pm 6
CEREBELLAR NUCLEI	93 \pm 12	76 \pm 8
CEREBELLAR HEMISPHERE	53 \pm 6	69 \pm 7
INF. COLLICULUS	184 \pm 31	97 \pm 7
SUP. COLLICULUS	89 \pm 15	90 \pm 10
SUBSTANTIA NIGRA	54 \pm 9	66 \pm 5

Correlation coefficient $r = 0.85$
 (By method of least squares)

putamen and the quantitative autoradiography data was obtained from these nuclei separately. The only brain region where my results were very different from the published data was the inferior colliculus, although I did, as others report, find this to be one of the most metabolically active regions studied. To judge the sensitivity of my method with the quantitative autoradiographic method, we can only compare my results with the findings of Engel et al (1978), who also examined amygdala kindled rats. These authors modified the autoradiographic method by killing rats only five minutes after injection of ^{14}C deoxyglucose, thus measuring only the rate of glucose uptake to cells and not the hexokinase reaction. In 5 kindled rats which developed seizures after deoxyglucose injection the changes in glucose consumption were very variable. In contrast to the consistent widespread and bilateral increases presented in Table 24, those authors showed that uptake was increased in the amygdala only ipsilateral to the electrode site in 2 rats, only contralateral to the electrodes in 2 rats and bilaterally in 1 rat. However, a bilateral increase in uptake was measured in substantia nigra, globus pallidus and bed nucleus of stria terminalis in all rats, in the ventrobasal nucleus of the thalamus and in the neocortex of 4 rats and the hippocampus of 3 rats. The consistent results reported here using a dissection procedure suggest that the method may be more discriminatory than the autoradiographic method in some hands. For example, Meibach (1977) directly compared an autoradiographic technique with the results of a dissection procedure in the rat brain. The dissection not only fully confirmed the autoradiographic findings, but extended them to areas not identified by quantitative autoradiography.

The available evidence, therefore, seems to suggest that a dissection procedure is certainly no less sensitive than autoradiographic methods for detecting small changes in the distribution of deoxyglucose at this macroscopic level. This of course does not detract from the value of autoradiography to obtain a complete scan of all brain regions or its potential for investigating deoxyglucose distribution at the cellular level.

2. Changes in the absolute amount of deoxyglucose GP accumulation caused by seizures

Table 26 shows the absolute amount of ^3H -deoxyglucose 6P expressed as cpm/mg. protein in the visual cortex following kindled seizures, electroshock seizures and in seizure-free controls. Kindled convulsions cause a 2.5-fold increase in glucose consumption and electroshock convulsions cause a 5-fold increase. It seems reasonable to suppose that this represents an increase in cerebral cortical GLYCOLYSIS during seizures. Bicuculline induced seizures in Wistar rats without an antihypertensive were accompanied by a 9-fold increase in cortical blood flow, which occurred simultaneously with the onset of seizures (Meldrum and Nilsson, 1976).

Pentylentetrazol induced seizures in the dog caused a 3.6-fold increase in cerebral blood flow (Plum et al, 1968) and in the monkey a 1.4-fold increase (Schmidt et al, 1945).

The results in Table 24 suggest that even in the presence of a large overall increase in cerebral blood flow regional differences ^{IN DEOXYGLUCOSE UPTAKE} are maintained.

Regional changes in glucose consumption during kindling and electroconvulsive shock are ^{NOT DUE TO} merely non-specific regional variations in blood flow as a consequence of the 3 - 5-fold increase.

Sokoloff (1977) has argued that deoxyglucose uptake relates to functional activity and not to blood flow, even though these may be closely related.

The results in Figure 19 show that in several brain regions deoxyglucose distribution is altered relative to unoperated controls in non-convulsed control groups of rats. This suggests that despite a large overall increase in glucose uptake regional increases due to specific local (possibly ictal) metabolic demands are accurately superimposed.

3. Relation between deoxyglucose distribution and electrographic ictal phenomena

Sokoloff (1977) has shown that glucose consumption is a measure of the functional activity of cells and during the discharge of an epileptic focus regional glucose consumption increases as expected for an energy requiring process. However, there is no reason to suppose that deoxyglucose distri-

bution should accurately reflect spiking activity as recorded by the electrocortecogram or depth electrodes, and examples have already been quoted where the firing rate of cells may be increased, but deoxyglucose accumulation is decreased. This may be explained by the following:

- a) Inhibition and excitation are both energy requiring processes. We do not know the relative energy requirement of the various components of neuronal functioning, but it seems likely that ion pump activity and neurotransmitter synthesis, release and re-uptake will be the source of major energy requirements. Release of inhibitory neurotransmitter with subsequent cell hyperpolarization may not be distinguishable from the release of an excitatory transmitter causing depolarization. Cell firing may not be the major determinant of energy consumption. (See examples given in the Introduction).
- b) Structures which show enhanced deoxyglucose uptake may be secondarily involved due to direct anatomical connections with an epileptic focus and may not be involved in the spread of discharge or sustain a discharge.

This technique describes functional relationships and not anatomical connections and the results should be complementary and not identical to techniques involving lesions or depth electrode recording used in the study of the spread of epilepsy.

4. The anatomy of kindling

From Figure 19 the following main conclusions can be drawn:

- a) The implantation of an electrode into the amygdala for a month causes significant changes in glucose utilization in the hypothalamus and septal nuclei.
- b) Animals previously kindled but not convulsed at the time of deoxyglucose injection do not differ from sham-operated controls.
- c) Partially kindled animals show increased glucose consumption bilaterally in the amygdala, hippocampus, and superior colliculus.

- d) Kindled (convulsed) animals showed increased glucose utilization in the amygdala, hippocampus, hypothalamus, septal nucleus, superior colliculus and substantia nigra.
- e) Electroshock convulsions caused increased glucose consumption in amygdala, hippocampus, hypothalamus, thalamus, striatum, septal nuclei, substantia nigra, cerebellar hemispheres, cerebellar nuclei, reticular formation.

These results support the notion that kindling from the amygdala involves specific, mainly limbic, pathways. Thus the effects of kindled convulsions are much less widespread than the effects of electroshock convulsions and in part involve the structures which are activated by partial kindling.

It is also noteworthy that the biggest changes in electroshock seizures occur in amygdala, hippocampus and hypothalamus, suggesting that these structures with low seizure threshold will participate in generalized seizures whatever the source of stimulus. That no relatively reduced levels of glucose consumption were measured vindicates the choice of the occipital cortex as a reference area. Even in generalized seizures the occipital and frontoparietal cortex (and the olfactory bulb) do not increase glucose uptake relative to other brain areas.

The bilateral nature of all the changes, even those observed in partially kindled animals, is in keeping with the observations of Kliot and Poletti (1979), who used the deoxyglucose autoradiographic method to measure metabolic changes associated with hippocampal after-discharge. Even after the initial unilateral hippocampal stimulus which evoked after-discharge, deoxyglucose uptake was increased in the hippocampus bilaterally. Wada and Sato (1974) showed electrographically that the contralateral amygdala is involved very early in the process of amygdaloid kindling.

The results of the deoxyglucose experiments are in keeping with the view that spread of a kindling evoked seizure from the amygdala follows specific paths to the brain stem, and the cortex, thalamus, striatum and cerebellum are not critically involved (see Section 1 - 6).

A long-held view about the role of the cerebral cortex in seizures was that the clonic elements of this were due to activity in the motor cortex and

tonic elements were subcortical, despite the observations of Pike and Elsberg (1925) that clonic convulsive seizures could be sustained in animals deprived of their cerebral motor cortex. Kreindler et al (1958) confirmed in rats and cats that stimulation applied to the region of the midbrain, reticular formation and periaqueductal grey matter evoked tonic-clonic seizures, which were entirely independent of cortical neurones. Moreover, when the MRF was stimulated during the progress of a cortically induced seizure, the brain stem attack supplanted the motor manifestations of the cortical discharge.

Gibbs and Gibbs (1936) had suggested two seizure systems, one was cortical and included the thalamus and basal ganglia, the other included the amygdala, fornix and parts of temporal cortex. The concept of generalized cortico-reticular epilepsy was introduced by Gloor (1968) to describe seizures in the pathogenesis of which cortical mechanisms were very important, but which also depended on thalamic and brain-stem reticular mechanisms for the development of bilateral spike and wave discharge.

Arana-Iniguez^{etal} (1954) and Gloor (1957) independently mapped the spread of after-discharge from the amygdala in cats and obtained similar results. The subcortical structures fired by an after-discharge from the amygdala were diffuse and extensive, extending from the septal area to the mesencephalon. Also involved were the hypothalamus and the hippocampus. The most constantly observed conduction was to the ipsilateral septal nuclei, hypothalamic and tegmental regions. The thalamus was less frequently involved and even during widespread subcortical seizures there was an absence of widespread involvement of the cortex, except for the anterior temporal and insular regions.

Mayanagi and Walker (1974) have stressed the very close functional relationship during seizure activity between the amygdala and the hippocampus. These usually develop seizure activity simultaneously and it was suggested that in terms of propagation they should be considered as a single complex. Krettek and Price (1977b) have provided anatomical evidence for such a close association.

It seems clear that the metabolic changes observed by the deoxyglucose method fit into the pattern of ictal spread established by neurophysiological

methods.

Collins et al (1976) measured changes in deoxyglucose in rats with a penicillin cortical epileptic focus. Mild motor jerks were associated with increased deoxyglucose in the ipsilateral cortex, striatum and thalamus and the contralateral cerebellum.

Generalized tonic-clonic seizures caused increased glucose use bilaterally in:-

Frontal cortex, thalamus, caudate/putamen, globus pallidus, substantia nigra, subthalamic nucleus, cerebellar cortex and lateral amygdala nucleus.

This pattern of deoxyglucose uptake, showing involvement of the thalamus, striatum and the cerebellum, is similar to my results obtained in electroshock induced generalized seizures and might be broadly classified as cortico-reticular in type. These results also contrast with my findings following amygdala stimulation. In this case, the changes I have measured in amygdala, hippocampus, hypothalamus and septal nuclei resemble the spread of after-discharge reported by Gloor (1957) and Arana-Iniguez et al (1954) from the amygdala, as recorded by depth electrodes.

The involvement of the superior colliculus was a constant feature of my experiments and an explanation for this is not available. It has been established that the rhythmic well-coordinated chewing movements, observed early in kindling and present in all of the partially kindled group of rats, are a feature of amygdala stimulation. The action is independent of the precentral motor cortex and involves direct activation from the amygdala of the trigeminal, facial glossopharyngeal and hypoglossal nuclei in the brain stem (Kaada, 1951). It could be conjectured that the superior colliculus - part of the visual system - is similarly stimulated directly from the amygdala.

CHAPTER 5

Ch. 5 GENERAL DISCUSSION

1. Biochemical changes which accompany kindling

A fairly wide range of biochemical derangements occur in the aftermath of convulsions and these may be the non-specific consequence of stress, hypoglycaemia hypoxia, hypercapnia, hypertension or other physiological change which accompanies seizure.

To be relevant in kindling any measured biochemical change should show a similar time course to the behavioural changes observed and should be compatible with other evidence independently arrived at as, for example, the effect of drugs on kindling. Biochemical changes are also more likely to be pertinent if they are specific and not one of a large number of changes. The long-lasting nature of kindling provides an excellent means for eliminating non-specific effects of the convulsions themselves, although clearly different processes may underlie several aspects of this form of epileptogenesis. It seems probably that mechanisms which sustain interictal spiking may differ from the processes which establish kindling during and immediately after the production of an after-discharge. It is usually these early processes which are studied by the pharmacological experiments described in (1 -9), but they probably differ not only from each other, but also from the long-term changes which maintain the epileptic focus almost permanently in kindling.

The available data is too limited to permit general conclusions about long-term neurochemical changes in kindling and the difficulties in discriminating between kindling and non-specific seizure effects are a problem with catecholamines and acetylcholine. Electroshock convulsions are followed by increases in monoamine postsynaptic responses (Modigh, 1975, Grahame-Smith et al, 1978) and after a series of convulsions some of these changes may be sustained (Fink, 1979).

Several types of process outlined in Section 1 - 10 may be the basis for lasting changes in neuronal excitability at an epileptic focus. The experiments presented in this thesis do support the view that lasting alterations in synaptic function do occur at the site of stimulation following amygdala kindling and because the changes are measured at least 4 weeks after a

convulsion they are unlikely to be non-specific sequelae of ictus. Thus the turnover rate of dopamine is increased in the stimulated amygdala after kindling. There is also a reduction in the basal level of cyclic GMP and a failure of cyclic GMP to respond to a variety of stimuli, including dopamine and haloperidol. In normal tissue slices these drugs induce a significant accumulation of the cyclic nucleotide.

The value of these observations is enhanced by the demonstration that no lasting change appears to occur in cholinergic activity nor in ATPase nor acetylcholinesterase activity at the site of stimulus.

2. The nature of a kindled focus

A body of biochemical evidence is emerging that lasting synaptic changes accompany kindling, but although several lines of evidence suggest changes in monoamine neurones, knowledge about these synaptic events remains scanty. A further question to be asked is whether these synaptic changes occur entirely in a population of cells at the site of stimulus, the effect of stimulus being to enlarge this primary focus by the recruitment of contiguous cells, or whether the spread of kindling reflects the breakdown by repeated bombardment of synaptic barriers between the site of stimulus and the motor systems. The latter model has been traditionally held since put forward by Racine (1972b, 1972c), but now several observations suggest the former explanation:

1. After-discharges from the hippocampus spread bilaterally and extensively to brain stem structures even after one or a few stimulations (Kliot and Poletti, 1979).
2. In my own findings using deoxyglucose, structures involved in Stage 5 kindled seizures were also involved in Stage 2 kindling without seizures, apart from substantia nigra, which could be involved secondarily as a result of motor activity. Likewise ECS increased activity in practically all of the structures involved in kindling. These observations suggest that seizure pathways or low threshold regions in the brain pre-exist kindling which utilizes, but does not create these paths of spread.

3. Collins (1978b) elegantly demonstrated the progressive enlargement of the tissue mass accumulating deoxyglucose at the site of stimulus in cortical kindling. He concluded that the main effect of repeated stimulation was to enlarge the focus rather than extend the spread.
4. Wise and Rackham (1979) showed that intraventricular injections to rats of β -endorphin caused limbic epileptiform activity which was not expressed as behavioural convulsions even though the pathways of limbic spread should have been enhanced by previous electrical kindling.

If this view is upheld, then kindling is subject to mass action and convulsions will occur only when a sufficient number of cells at the stimulus site are activated. Biochemical changes would then be expected to occur principally at the 'primary' and perhaps contralateral focus, but not necessarily along the proposed route of spread. At the physiological level it is intriguing that McNaughton (1979) observed that long-lasting synaptic potentiation, but not short-term potentiation, was a cooperative process dependent on the number of fibres stimulated. Long-term potentiation would only develop if sufficient fibres were fired by one stimulus.

At the clinical level Wilkins et al (1979) have proposed a mass action effect in pattern sensitive epilepsy so that ictus only develops when a critical number of sensitive cells in the striate cortex is activated by a sensory input.

A strong argument against the 'mass action' model of kindling and in favour of kindling spread by means of progressive facilitation of synapses along a set pathway has been the observation that the 'transfer effect' is maintained between amygdalae in split brain animals. This would suggest that the after-discharges from the second amygdala to be stimulated can connect into brainstem paths already facilitated by the kindling of the first amygdala.

However, if further experiments show that the 'transfer effect' is artefactual and merely due to the presence of an electrode, then this objection to the 'enlarging focus' model of kindling is removed.

Amygdaloid kindling shows several intriguing parallels with human temporal lobe epilepsy. It is well established that the surgical removal of a discreet epileptic focus in the human temporal lobe can effectively and permanently prevent the occurrence of further fits. A model of kindling which had as a basis the enlargement of a focus at the site of stimulus rather than the progressive facilitation of synapses to distant structures would best fit with this aspect of the human disease.

3. The clinical relevance of kindling

(a) Kindling and epilepsy in man:

A major aetiological factor in temporal lobe epilepsy is the occurrence of childhood febrile convulsions, but a puzzling feature of the relationship between the two is the enormously long time-lag from the time of seizures and presumed brain damage at the age of 1 - 2 years and the onset of temporal lobe attacks which may sometimes become manifest only after puberty. Once fits develop they may persist for the life-time of the sufferer. Although human beings may be extremely resistant to kindling stimulation, it remains a possibility that the type of plastic change which underlies experimental kindling could also develop in response to the minimal temporal lobe pathology underlying temporal lobe epilepsy.

Post-traumatic epilepsy may also develop following a prolonged 'incubation' period. About 70% of cases of post-traumatic epilepsy develop within two years of injury. The remaining 30% of cases may not become manifest for 5 - 10 years, or in exceptional cases even longer still (Jennett, 1978). The cause of post-traumatic epilepsy is entirely unknown, but it is tempting to speculate again that a kindling process may develop in response to intracerebral haematoma or other lesions consequent on injury.

A motive for further research into the mechanism of kindling is the hope that these major causes of clinical epilepsy may be preventable or perhaps reversible.

It may be impossible to confirm a kindling mechanism in human epilepsy, but it is possible to examine for a change in the incidence of epilepsy in patients who have received a variety of spaced brain stimulations.

In a recent survey (Blackwood et al, submitted for publication) a group

of 166 patients aged 21 - 69 years who had received electroconvulsive therapy more than 18 months previously were interviewed and the incidence of epileptic fits following ECT was measured.

The group had received a mean of 16.8 treatments (Range 1 - 75), given in 1 - 14 courses, sometimes over several years.

The occurrence of spontaneous epileptic fits following ECT in this group did not exceed the number expected for the community as a whole.

(b) Kindling, addiction states and the psychoses

Post and Kopanda (1976) have compared the superficial resemblances which exist between the enhanced response to certain drugs, such as amphetamine and cocaine, experienced by regular users with changes observed in the kindling phenomenon. It is a well known clinical observation that repetitive administration of these stimulant drugs, which at first act merely as euphorants, may in certain patients lead to progressive alteration in behaviour and sometimes cause a paranoical psychotic reaction. Furthermore, regular amphetamine users may experience an immediate activation of a psychotic state even after a prolonged abstinence from the drug. It is proposed that this reverse tolerance effect is due to pharmacological kindling in no way connected with seizure processes, but involving brain regions critical for other aspects of behaviour and mood. Taking the argument a step further it was proposed that the 'stimulus' necessary to render a person susceptible to psychotic episodes, either of a schizophrenic or affective nature, might be the repeated trauma of stressful life events at critical developmental periods (Meyersburg and Post, 1979).

As with much of interpretive analytical psychiatry, this proposal is probably untestable. However, the role of the mesolimbic dopaminergic system in schizophrenia and the possibility that this or other neurotransmitter systems may be modified in a permanent way by a kindling type of stimulus (Stevens and Iivormore, 1978, Lamprecht, 1977) is an interesting proposition and may be a fruitful line of research into the pathology of the psychoses. It may also be useful to consider a process of pharmacological kindling operating in alcohol withdrawal states (Ballenger and Post, 1978).

(c) Kindling and therapeutic brain stimulation

Although kindling in man remains speculative, there is justification for the caution expressed by Goddard (1971) in the use of chronic intracranial electrodes in man. Despite the confidence of Delgado (1977) about the safety of this procedure, a single clinical report (Sramka et al, 1977) has suggested that epilepsy may develop as a result of repeated electrical stimulation applied to the thalamus for the relief of pain.

Furthermore, a recent review of a rather bizarre series of patients is also relevant in this context. In some societies the insertion of intracranial sewing needles in infants before the closure of the fontanelles is forensically extremely difficult to detect and is a well documented practice for achieving infanticide. Abbassioun et al (1979) discussed a series of patients who have survived attempted infanticide by stainless steel needles. Headaches and epilepsy are the main symptoms and symptoms often first appeared only when the patient had reached adulthood. The interval between the assumed age of the patient at the time of needle insertion and the onset of epilepsy varied from 18 - 24 years except in the case of a 43-year old man who had developed convulsions in infancy. These unusual clinical cases strongly support the view that chronic metal implants may be epileptogenic in man. Further studies on kindling must take into account the effects of the presence of electrodes in addition to the effect of any stimulus applied via these electrodes.

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